ARCHIVES OF PATHOLOGY

OCTOBER 1945

CONTENTS OF PREVIOUS NUMBER

SEPTEMBER 1945. NUMBER 3

EFFECT OF EXCESSIVE INGESTION OF SODIUM CHLORIDE ON THE CHICK, WITH PARTICULAR REFERENCE TO RENAL CHANGES

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During the early months of 1943, heavy losses among 10 to 20 day old chicks were reported from many parts of the island of Puerto Rico. On some poultry farms the mortality ranged from 50 to 100 per cent. The affected birds presented marked ascites, with an enlarged, tense, translucent abdomen, hydropericardium and a variable amount of subcutaneous edema. In all instances the poultry farmers concerned had recently purchased a new lot of feed (PR1) from a local dealer. This feed, though of poor physical quality, should theoretically, in view of its ingredients as given by the dealer,[1] have been adequate for good growth.

Preliminary observations were made to determine the cause of the reported high mortality among commercially reared chicks and to explain, if possible, the mechanisms involved.

PRELIMINARY EXPERIMENTS

Twenty-one day old New Hampshires were fed the suspected food mixture and given water ad libitum. In 6 of them anasarca developed, and these died or

1. The composition is stated to be as follows:

	Pounds per Ton
Rice bran	50
Ground yellow corn	500
Wheat middlings	500
Alfalfa meal	100
A vitamin concentrate containing riboflavin, pantothenic acid, nicotinic acid, choline and vitamin D	1
Peanut meal cake	150
A vitamin D preparation in oil containing not less than 10,000 U. S. P. units per gram	1
Minerals	69
Bone meal	5
Sodium chloride	5

were killed between the third and the eleventh day. Eight others that died or were killed before the thirty-ninth day had no edema. To the diet of the 6 remaining birds sodium chloride was added to the extent of 5 per cent of the feed on the forty-second day. One bird died with hydrops two days later, while, of the 5 survivors at the end of the week, 3 showed ascites.

In view of the observations of Dam and Glavind [2] and Bird and Culton [3] that vitamin E deficiency may be associated with generalized edema in the chick, it was thought that this might be an instance of such deficiency. Accordingly, 30 New Hampshires 1 day old were divided into two groups of equal size. Both lots were maintained on the new feed. Each chick in one of the groups was given a daily oral dose of 1 mg. of racemic alpha tocopherol. Subsequently this dose was doubled. Because of the observations of Bird [4] that the addition of salt to vitamin E–deficient diets accelerated and enhanced the development of edema, sodium chloride (2 per cent by weight) was added to the feed of both groups of chicks on the eighteenth day of the experiment. Seven days later this amount of salt was doubled. The last survivor was killed on the sixth day of the last-mentioned salt level. Of the 15 chicks that were treated with alpha tocopherol, 9 with demonstrable hydrops died or were killed; 3 of the treated birds had been given the commercial feed alone, 4 this with the additional 2 per cent sodium chloride, and 2 the feed with the additional 4 per cent sodium chloride. Of the untreated 15 birds, 10 presented hydrops, 2 on the diet alone, 4 with additional 2 per cent salt and 4 with additional 4 per cent salt.

It was evident, therefore, that vitamin E deficiency was not the factor involved in the production of the edema. This was further substantiated by feeding PR1 to female rats during pregnancy and lactation (rats previously reared and maintained on a diet low in vitamin E). Viable litters were obtained which survived to the weaning period without the development of muscular dystrophy. Furthermore, encephalomalacia never developed in the chicks on this diet.

Our attention was then turned to the possible significance of the salt content of PR1. Chemical analysis revealed that this feed contained 2.87 per cent of sodium chloride. A newer feed mixture put up by

2. Dam, H., and Glavind, J.: Nature, London **142**: 1077, 1938; **143**:810, 1939; Skandinav. Arch. f. Physiol. **82**:299, 1939.

3. Bird, H. R., and Culton, T. G.: Proc. Soc. Exper. Biol. & Med. **44**:543, 1940.

4. Bird, H. R.: Science **97**:98, 1943.

the same dealer (PR2[5]) contained 0.52 per cent sodium chloride.

When the constituents of PR1 were obtained and mixed in the proportions stated by the dealer, the sodium chloride content of the mixture was 0.25 per cent. This feed mixture is designated as PR3.

A fourth diet (PR4) was prepared to determine whether or not PRI was quantitatively or qualitatively deficient in protein. This diet contained 3 parts of PR1 and 1 part of powdered skimmed milk. The sodium chloride content of the powdered milk was 1.88 per cent; that of the total feed mixture, 2.6 per cent.

Thirty 1 day old New Hampshires were divided into four groups and fed these four diets, respectively. To all feeds sodium chloride was added in the concentration of 2 per cent after the tenth day, and seven days later the sodium chloride content was increased to 4 per cent. The additional sodium chloride was employed for the same reason as in the previous experiment, viz., that it would help to make manifest more readily any latent tendency of the diets to produce edema. The results were as follows:

PR1—Hydrops developed in 5 of 6 birds. None of these was fed on the diet alone; 4 were given PR1 with the addition of 2 per cent salt and 1 PR1 with the addition of 4 per cent salt.

PR2—Hydrops developed in 6 of 6 birds. None was fed the diet alone; 1 was given PR2 with the addition of 2 per cent salt and 5 PR2 with the addition of 4 per cent salt.

PR3—Hydrops developed in 9 of 12 birds. None was fed the diet alone; 1 was given PR3 with the addition of 2 per cent salt and 8 PR3 with the addition of 4 per cent salt.

PR4—Hydrops developed in 5 of 6 birds. One bird was fed the diet alone; 3 were given PR4 with the addition of 2 per cent salt and 1 PR4 with the addition of 4 per cent salt.

It was apparent from these results that with the feeds PR1 and PR4, originally high in salt, the maximal incidence of hydrops occurred when 2 per cent sodium chloride was added, whereas with the other two diets, PR2 and PR3, originally low in salt, the maximal incidence of hydrops occurred when 4 per cent sodium chloride was added. This clearly indicated that the edema-producing factor in the commercial feed was primarily its excess of salt. Since commercial feeds for chicks contain on the average 0.5

5. The composition of PR2 was given as follows:

	Pounds per Ton
Corn meal	450
Alfalfa	200
A vitamin concentrate containing riboflavin, pantothenic acid, nicotinic acid, choline and vitamin D	100
Wheat bran	50
Veal scraps	100
Dried blood	40
Bone meal	10
A vitamin D preparation in oil containing not less than 10,000 U. S. P. units per gram	1
Peanut meal	50

to 1.0 per cent of sodium chloride but may even contain as high as 2 per cent, the physical quality of PR1 appeared to be a contributing factor. This was borne out to a certain extent by feeding leached PR1 to 1 day old New Hampshires and white Leghorns. These grew poorly. In many of the white Leghorns ataxia and tremor developed. The mortality in general was high. Whole liver or yeast added to the leached food improved the growth of the chicks.

This part of the problem was no longer pursued, however. Our attention was turned to the effect of excessive salt intake on chicks.

FURTHER EXPERIMENTS

Experiment 1.—In order to observe the effect of an excessive intake of salt on recently hatched chicks, 18 chicks 2 days old of a mixed breed of New Hampshire and Rhode Island reds were fed PR2 to which 3 per cent sodium chloride had been added (total salt content, 3.5 per cent). A second group, 11 birds (controls), grew normally on PR2 alone.

Experiment 2.—Thirty-two 3 week old New Hampshires were maintained on a commercial feed of unknown composition and salt content but which allowed for good growth and development of the controls. These birds were divided into three lots. One lot of 11 served as controls. A lot of 15 had 3 per cent sodium chloride added to their food. A lot of 6 was retained on the diet alone but was given 0.9 per cent saline solution to drink. The food and water consumed daily by each lot were determined. As in all the previous experiments, the birds were weighed once or twice weekly. Many of the birds were killed or died at various intervals during the first three weeks of the experiment. At the end of that period 3 chicks that had been given the 3 per cent salt admixture were returned to the normal diet and killed after three, ten, and twenty-eight days, respectively. The remaining birds given the 3 per cent salt admixture and their controls were allowed to live for three or more months longer.

Since the determinations of the food and water consumed by the lots failed to provide accurate information on the amounts consumed by the individual birds and were influenced by the failure of sick birds to eat or drink normally, this experiment was repeated and designated experiment 3.

Experiment 3.—Twenty-eight 3 week old New Hampshires and white Leghorns, reared in the laboratory, were maintained on the same commercial feed as the birds used in the previous experiment but were housed in separate pens. Nine birds served as controls. Eight were given 0.9 per cent saline solution to drink, and the remaining 11 birds were fed the commercial diet plus 3 per cent sodium chloride. Of these 11 chicks, 6 were killed three, six, nine, twelve, twenty-four, and thirty-three hours, respectively, after having been placed on the diet containing salt in excess. All other birds were allowed to survive for six weeks, when the experiment was terminated. The amounts of food and water consumed daily by the individual birds were recorded.[6] All birds were weighed thrice weekly.

6. The determination of water consumption was perforce not entirely accurate. Bottles with a wide

At intervals in the course of each of these experiments, blood was analyzed for total plasma protein and sodium chloride. The protein content of ascitic fluid was also determined. The protein was determined by the micro-Kjeldahl method. In the later experiments hematocrit readings were recorded in most instances. All birds that died or were killed were examined, and the organs were weighed and sectioned for histologic study.

RESULTS

It will be seen from the data recorded in table 1 that a salt intake at a higher level (3.5

TABLE 1.—*Experiment 1. Effect of an Excessive Intake of Salt on Newly Hatched Chicks*

				Survival Period						
Days.....	2	3	5	6	7	8	10	14	17	20	34
Birds.....	1°	1	1°	1†	1	1	6†	3†	1	1	1

° No hydrops developed.
† One bird with advanced hydrops was killed.

per cent) than that of the original edema-producing commercial feed (2.78 per cent) has a devastating effect on newly hatched chicks. In 16 of the 18 birds anasarca developed. Except for the 3 that were killed because of advanced hydrops, all died between the second and the thirty-fourth day. The peak of mortality was reached on the tenth day.

By contrast, in the second experiment, the exposure of 3 week old chicks to a similar concentration of salt in the food resulted in the death of 6 of 15 birds. All of these exhibited varying degrees of hydrops. Two birds died on the second day, 1 on the third, 1 on the sixth and 2 on the seventh. One bird was killed on the seventh day because of marked hydrops. In the remaining 8 birds no appreciable edema was present either during life or at the time of autopsy. Of the 11 birds similarly treated in the third experiment, slight edema was observed in 1 killed after twelve hours. Somewhat more marked edema was present in a second bird, killed after twenty-four hours, and it was quite marked in a third chick, put to death after thirty-three hours. Three birds killed at three, six and nine hours showed no pathologic changes. Of the remaining 5 birds, demonstrable edema developed in only 1 during the next six weeks, and this chick had none at all at the time of autopsy.

enough opening were used to allow the bird to dip its beak. A certain amount of water was therefore lost by the wetting of the skin about the beak and the sprinkling of water by lateral movements of the bird's head following the act of drinking. Losses as a result of evaporation were, however, corrected for.

Saline solution (0.9 per cent) as drinking water was almost as drastic in its effect on 3 week old birds as was the food mixture containing 3.5 per cent sodium chloride on newly hatched chicks. Combining the results of the last two experiments, we find that, of the 14 birds, 3 died with hydrops three, five and eight days, respectively, after the onset of the experiment. Four were killed because of advanced hydrops after four, seven, fifteen and twenty-one days, respectively. Two were killed at the end of the first week because of the development of neurologic disturbances that were apparently unrelated to the salt intake, since a similar syndrome appeared in an occasional control. One of these had no edema; the other had hydropericardium only. Two were killed on the eighth and the twenty-second day, respectively, and showed mild edema. Only 3 survived beyond the third week. These showed little or no edema at autopsy.

OBSERVATIONS

Objective Evidences of Diseases in Birds Receiving Salt in Excess.—While ascites in recently hatched chicks was readily apparent, it was difficult to determine its presence in older birds unless it was quite advanced. Fluid accumulations in subcutaneous spaces occurred chiefly in the loose skin about the neck and the ventral aspects of the thorax and the abdomen. Dyspnea occurred in birds at rest or after handling, whereas gaping was more often seen to occur after excitement. These signs, particularly the latter, were sometimes unaccompanied by obvious anasarca. Frequently, however, they were found at autopsy to be associated, in the absence of hydrops elsewhere, with hydropericardium. Cyanosis was rarely observed. A watery type of "diarrhea" was a constant feature. Birds with edema had an unhealthy appearance. Their feathers were ruffled, and they were often droopy and listless. Death in some instances occurred suddenly with convulsions. In other instances a shocklike syndrome supervened, which sometimes lasted for a day or more before death occurred.

Laboratory Findings.—In general the total plasma proteins were lower in the experimental birds without edema than in the controls. It is evident from table 2, however, that in the presence of edema there was a sharp reduction in total plasma proteins, sometimes to as much as 50 per cent or more of the normal values. The protein content of the ascitic fluid was unexpectedly high and often approached that of the plasma. The plasma chlorides were appreciably elevated, particularly in those birds that were given 0.9 per cent saline solution to drink. The hematocrit readings were generally lower for nonedematous experimental birds than for the controls and were markedly reduced for the edematous ones. This was true in a larger series of birds than that recorded in the table.

Food, Water and Salt Intake and Body Weight.—The amounts of food and water consumed by the newly hatched chicks which were fed excess salt were not determined. It will be noted from table 3, however, that their average gain in weight was less than

that of the controls. Actually it was probably much less, as many of the experimental birds were edematous at one time or another.

The daily estimation of the food and the water consumed by groups of chicks in the second experiment yielded results essentially comparable to those obtained for individual birds in the third experiment. The results for the latter are listed by weeks in table 4.

It is apparent that birds ingesting excessive amounts of salt drink large amounts of water—in fact, three or even four times the amount consumed by the controls. The water consumption of the controls and of the group given the food mixture containing 3 per cent salt bore a close relation to the food intake. It will be noted that among the controls the ratio of water to food intake varied within fairly strict limits for any one bird. For the group generally the water intake ranged from 1.5 to 4.5 cc. per gram of food. The

on the amount of food ingested, a fairly constant amount of water being imbibed to dilute the additional salt to subphysiologic concentrations. With added salt in the form of 0.9 per cent saline solution, however, the quantity of water consumed is unrelated to anything but the maximum amount which the bird can drink in attempting to satisfy its thirst. The lower intake during the first week in some birds was probably due to their distaste for the salty water.

While a certain amount of reduction in the consumption of food occurred in some of the birds during the first few weeks of the 3 per cent salt–food mixture, as compared with the controls, it was never marked except in those that were edematous and evidently unwell. With 0.9 per cent saline solution as drinking water, a marked reduction of food consumption was the rule in the first few weeks, and the intake of food

TABLE 2.—*Values of Total Plasma and Ascitic Fluid Proteins and Plasma Chlorides and Hematocrit Readings for Chicks Receiving Different Concentrations of Sodium Chloride in Feed or Water*

Chick	Sex	Age, Days	Diet	Percentage of Sodium Chloride (NaCl)	Duration, Days on NaCl	Presence of Hydrops	Total Proteins, Gm. per 100 Cc.		Wintrobe Hematocrit Reading, Uncorrected	Blood Chlorides as NaCl, Mg. per 100 Cc.
							Plasma	Ascitic Fluid		
O*	F	1	....	....	..	..	1.21	....	....	...
O	M	1	....	....	..	..	1.13	....	....	...
39	F	6	PR1..........................	2.87	5	+	0.63	0.50	....	...
41†	M	7	PR1..........................	2.87	6	+	0.94	0.58	....	...
42	M	8	PR1 and alpha tocopherol	2.87	7	+	0.88	0.69	....	...
43	F	8	PR1 and alpha tocopherol	2.87	7	+	1.19	0.59	....	...
45	M	9	PR1 and alpha tocopherol	2.87	7	+	0.88	0.55	....	...
O	M	15	PR2..........................	0.52	..	..	1.44	....	....	...
C	M	22	PR2..........................	0.52	..	..	1.88	....	....	...
C	M	22	PR2..........................	0.52	..	..	3.81	....	....	...
48	F	20	PR1 and 2% NaCl..........	4.87	18	+	1.44	Not determined	....	...
50	F	24	PR1 and alpha tocopherol and 2% NaCl	4.87	23	..	1.31	....	....	...
52	F	24	PR1 and 2% NaCl..........	4.87	23	+	1.06	1.00	....	...
53	F	24	PR1 and 2% NaCl and alpha tocopherol	4.87	23	+	0.99	0.88	....	...
54	F	25	PR1 and 2% NaCl..........	4.87	24	..	1.81	....	....	500
56	F	25	PR1 and 2% NaCl..........	4.87	24	+	1.28	0.64	....	560
O	M	26	Commercial................	?	..	..	2.94	....	31.5	540
C	M	34	Commercial................	?	..	..	2.25	....	25.0	500
C	M	34	PR2..........................	0.52	..	..	2.69	....	29.0	...
140	F	26	Commercial and 0.9% saline drinking water	....	4	+	0.82	0.57	14.0	660
146	M	29	Commercial and 0.9% saline drinking water	....	7	+	0.88	0.31	16.5	670
149	M	30	Commercial and 0.9% saline drinking water	....	8	Slight	1.31	....	22.0	640
159	F	43	Commercial and 0.9% saline drinking water	....	21	..	1.38	....	26.5	...
147	F	29	Commercial and 3% NaCl.	3.00	7	+	1.56	1.31	28.5	560
151	F	34	Commercial and 3% NaCl.	3.00	12	..	1.69	....	23.0	685

* O represents a control.

† Edema fluid from the thigh contained 0.94 Gm. total proteins per hundred cubic centimeters.

TABLE 3.—*Experiment 1. Average Body Weight in Grams for Experimental and Control Groups*

Days..........................	4	8	12	17	21
Controls......................	53.5	69.7	91.8	132.2	151
Experimental chicks..........	46.0	68.3	87.3	95.0	104

group variation for birds receiving an added 3 per cent salt was from 3.7 to 6.1 cc. of water per gram of food, with again a narrow range of variation for any single bird. In the group of birds that were given 0.9 per cent saline solution to drink the relationship was not nearly so close. There was wider variation in water consumption not only among individual birds but in the group as compared with the other two, viz., from 3.5 to 11.3 cc. per gram of food. This difference is due to the fact that the excessive consumption of water in birds with salt added to the food is dependent

rose to higher levels only in the few birds that survived the earlier experimental period.

These differences in food consumption were reflected in the growth curves of the birds. Those on the 3 per cent salt–food mixture grew fairly normally once they compensated for the excessive intake of water and were able to ingest a sufficient amount of food to permit growth. In the birds receiving excess salt as 0.9 per cent saline solution growth was poor, or there was an actual loss of weight preceding death. In a few compensated survivors the growth curve approached the normal but was at a lower level.

The immense strain placed on the chick's excretory and circulatory mechanisms becomes apparent if one considers the amount of salt over and above that contained in the commercial feed that was ingested by the birds each week. In those on the 3 per cent salt–feed mixture it increased from an average of 6.8 Gm. for the first week of the experiment to 12.6 Gm. for

TABLE 4.—*Weekly Gain in Body Weight of Chicks in Experiment 3, Together with the Amounts of Food, Salt and Water Consumed*

Chick	Sex	Week	Body Weight, Gm.	Weekly Gain in Body Weight, Gm.	Food, Gm.	Weekly Intake of Salt in Addition to That of Diet, Gm.	Weekly Intake of Water, Cc.	Amount of Water per Gm. of Food, Cc.	Range of Daily Water Intake as Percentage of Body Weight
Control group:									
204	F	1	184	...	228	....	382	1.6	14.5-37
		2	282	98	343	....	574	1.6	
		3	372	90	361	....	554	1.5	
		4	456	84	374	....	573	1.5	
		5	576	120	427	....	647	1.5	
		6	654	78	447	....	677	1.5	
209	F	1	188	...	208	....	350	1.7	14.1-31.3
		2	266	78	272	....	473	1.7	
		3	338	72	294	....	507	1.7	
		4	404	66	366	....	588	1.6	
		5	510	106	390	....	665	1.6	
		6	574	64	425	....	670	1.5	
207	M	1	187	...	213	....	393	1.8	18.3-38.5
		2	305	118	331	....	643	1.9	
		3	431	126	415	....	855	2.0	
		4	540	109	509	....	1,191	2.3	
		5	662	122	551	....	1,239	2.2	
		6	708	46	519	....	1,130	2.1	
214	M	1	184	...	177	....	321	1.8	11.4-29.7
		2	273	89	252	....	456	1.8	
		3	371	98	300	....	515	1.7	
		4	414	43	330	....	581	1.7	
		5	526	112	369	....	603	1.7	
		6	575	49	381	....	853	2.2	
Group with 3 per cent salt in its food:									
203	F	1	226	...	222	6.6	900	4.0	36.5-60.7
		2	332	106	280	8.4	1,200	4.2	
		3	411	79	318	9.5	1,256	3.9	
		4	463	52	385	11.5	1,631	4.2	
		5	580	117	364	10.9	1,579	4.3	
		6	638	58	477	14.2	2,146	4.5	
210	M	1	235	...	219	6.5	827	3.7	30.2-54.7
		2	343	108	284	8.5	1,070	3.7	
		3	450	107	327	9.8	1,237	3.7	
		4	482	32	355	10.6	1,391	3.9	
		5	635	153	429	12.8	1,670	3.8	
		6	651	16	391	11.7	1,527	3.9	
213	M	1	238	...	236	7.0	980	3.9	36.1-63.4
		2	330	92	272	8.1	1,120	4.1	
		3	432	102	297	8.9	1,234	4.1	
		4	514	82	299	11.9	1,685	4.2	
		5	661	147	421	12.6	1,920	4.5	
		6	725	64	451	13.5	1,999	4.4	
208	F	1	239	...	178	5.3	910	5.1	41.7-61.0
		2	252	13	179	5.3	997	5.5	
		3	336	84	225	6.7	1,076	4.7	
		4	400	64	298	8.9	1,414	4.7	
		5	529	129	345	10.3	1,605	4.6	
		6	558	29	372	11.1	1,749	4.6	
206	F	1	...	...	285	8.5	1,230	4.3	58.6-75.0
		2	393	...	332	9.9	1,556	4.6	
		3	477	54	362	10.8	1,895	5.2	
		4	552	105	427	12.8	2,561	5.9	
		5	714	162	520	15.6	3,200	6.1	
		6	819	105	586	17.5	3,423	5.8	
Group given 0.9 per cent saline solution as drinking water:									
188	F	1	160	...	96	7.9	887	9.2	85.5-92.5
			Died with hydrops						
189	F	1	177	...	103	7.2	801	7.7	55.6-68.9
			Died with hydrops						
205	F	1	250	...	196	10.0	1,118	5.7	61.0-120.8
		2	307	48	182	16.9	1,878	10.2	
		3	380	73	230	20.4	2,274	9.8	
		4	419	39	301	25.1	2,790	9.2	
		5	593	174	374	26.7	2,975	7.9	
		6	610	17	447	32.5	3,616	8.0	
191	F	1	298	...	194	10.8	1,202	6.1	59.3-73.7
		2	340	42	131	13.4	1,489	11.3	
		3	263	−77	113	11.0	1,227	10.8	
			Died with hydrops						
201	F	1	251	...	168	10.4	1,157	6.8	42.0-77.6
		2	296	47	167	10.8	1,211	7.2	
		3	339	41	191	11.8	1,319	6.9	
		4	411	72	256	14.8	1,648	6.4	
		5	494	83	296	15.4	1,717	5.8	
			Killed						
190	F	1	235	...	200	6.3	707	3.5	36.1-98.2
		2	345	110	185	13.2	1,856	7.3	
		3	292	−53	152	15.0	1,670	10.9	
			Died with hydrops						

the sixth week, whereas with 0.9 per cent saline solution as drinking water it increased from 8.7 Gm. for the first week to 14.6 Gm. for the third week and to 32.5 Gm. in the sole survivor at the sixth week. While the weekly fluid intakes increased in all birds during this growth period, it is of interest to note the amount of water ingested in any one day in relation to the body weight on the same day. In the controls this varied from a maximum of 38.5 per cent of the body weight to a minimum of 11.4 per cent, with the trend from the higher to the lower value corresponding to the increased growth of the bird. The normal bird from 3 to 5 weeks of age drinks a daily quantity of fluid equivalent to one fourth or one third of its body weight. This ratio falls slowly with individual variations to about one sixth or one ninth of the body weight at the end of nine weeks. In birds on the diet with 3 per cent salt this variation was anywhere from 75 to 30.2 per cent, and in those drinking 0.9 per cent saline solution it was 120.8 to 36.1 per cent; i. e., there were days when the bird drank a quantity of fluid the weight of which was equivalent to or greater than its own body weight. This is all the more impressive when one considers that the body weight of a bird with a high salt intake includes excess interstitial fluids aside from the more prominent collections of fluid in serous cavities or subcutaneous spaces.

Gross Changes Observed at Autopsy.—The distribution of the edema fluid was not uniform. In some of the affected birds marked subcutaneous edema was associated with little fluid in the serous cavities, or marked ascites was present with little subcutaneous edema. In general, however, a more constant finding was an increased amount of pericardial fluid. This was observed in birds with absence of edema elsewhere and even in older, well compensated birds, showing neither dyspnea nor gaping. The maximum amount of ascitic or pericardial fluid varied with the size of the bird. Five to 10 cc. of ascitic fluid was generally found in recently hatched experimental chicks; 25 cc. in 20 day old chicks; 40 cc. in 36 day old ones; 100 cc. in 1 bird that was 78 days old and had been on the 3 per cent salt–food mixture for thirty-six days, and 210 cc. in a 121 day old bird that had been given salt on an increasing scale up to 15 per cent of the diet for fifty-one days. The amount of pericardial fluid varied from a maximum of 0.95 cc. in recently hatched chicks to as much as 40 cc. in the oldest 2 birds just described. The serous fluids were clear and either light or deeper yellow. Some specimens contained coagulums or clotted rapidly on aspiration. Cultures were uniformly negative. Because the avian peritoneal cavity is divided into three compartments by the suspensory ligament of the liver and the right and left coronary ligaments, occasional accumulations of fluid were found in only one peritoneal division. With long-standing persistent ascites, secondary changes were noted in the right lobe of the liver, capsular thickening and nodularity, which resulted presumably from pressure of the fluid in the poorly distensible right upper abdominal compartment.

The lungs were often edematous, particularly in birds showing generalized anasarca. The kidneys were pale and edematous when ascites was present but otherwise, except perhaps for vascular accentuation, were not remarkable. In recently hatched experimental chicks, however, small renal cysts were found often in association with the edema. Except for testicular

swelling in an occasional cockerel with ascites, the viscera were normal, underdeveloped or atrophic, depending on the development and the nutritional status of the experimental chick.

With prolonged survival, most birds ingesting excessive salt, whether compensated or decompensated, had a variable degree of cardiac hypertrophy. This was sometimes extraordinary. There was also some degree of renal hypertrophy. In order to establish more exactly the degree to which cardiorenal hypertrophy develops, it will be necessary to employ a larger number of birds and to standardize conditions to a greater extent than has been possible thus far.

Microscopic Observations.—Except for retarded myocardial development and persistence of myelopoiesis in some of the recently hatched experimental chicks, there were no unusual features in the heart. Mitoses occurred in myocardial fibers in general with the same frequency in the experimental birds as in the controls in the first four to six weeks of life. Subintimal hemorrhages were noted in some arterial vessels on rare occasions. The edema of the lungs was largely interstitial, occurring around larger bronchial and vascular radicles as well as interlobularly. The interlobular edema was often sufficiently extensive to compress the lobular units of the lung. There was no visible fluid in the air spaces, however. In regard to the liver, capsular thickening and subcapsular necrosis and fibrosis were occasionally observed in those birds with prolonged ascites. No lobular reorganization or hyperplasia accompanied these changes. Atrophy of the liver cells varying in accord with the nutritional status was found. Testicular enlargement, when present, was caused by interstitial edema and also, as Selye[7] pointed out, by dilatation of the seminiferous tubules. There was almost invariably a greater amount of hemopoietic tissue in the marrow of the experimental birds than in that of the controls. With the exception of the kidneys, the other organs were not remarkable.

In the kidneys, the principal changes were observed in the glomeruli. In order to understand these, it is perhaps well to recapitulate briefly present knowledge of the normal histologic structure of the avian glomerulus (figs. 1 and 3). This structure is made up essentially of a nucleated core, over which run several branching capillaries. The filtering surface of the capillary is covered by a basement membrane, to which prominent visceral epithelial cells are attached. Opposite the filtering surface the capillary is anchored to the core by delicate collagenous or reticular fibers, which tend to be more condensed and prominent where the basement membrane and the core are apposed between the capillaries. These fibers run through the core to join those at the hilus about the afferent and efferent arterioles. The nuclei of the core are separated by a small amount of substance which stains with eosin or aniline blue but which is not uniformly argyrophilic and does not show definite fibrillar structure. Cell boundaries are not definable. It is for this reason that some investigators have regarded the core as being syncytial in character. Vilter[8] has recently restudied and reviewed this subject and as a result of his observations on the morphogenesis of the avian glomerulus has concluded that the core is multicellular, of fibroblastic character, with collagen separating the individual cells.

7. Selye, H.: Canad. M. A. J. **47**:515, 1942.
8. Vilter, R. W.: Anat. Rec. **63**:371, 1935.

In the developing chick there is nephrogenic tissue subcapsularly and interlobularly for the first three or four months, which provides for an increasing number of glomeruli in the postnatal period. With growth and development of the bird, the glomeruli increase in size, differing, however, in general from the mammalian glomeruli, in that those in the juxtamedullary areas are by far the largest. These stand out in sharp contrast to the appreciably smaller ones in the peripheral portions of the cortex. In the latter zones there is a certain amount of gradation in size of the glomeruli, the smallest ones being found subcapsularly. This relationship apparently parallels the anatomy of the interlobular cortical arteries, which are quite large near the medulla but rapidly decrease in size peripheralward. Volume and rate of blood flow are hence apparently greatest in the glomeruli of the inner portion of the cortex. The normal growth of the glomeruli in the outer portions of the cortex involves an increase in the size of the core and the number of its nuclear elements with a corresponding increase in the length of the overlying capillaries. While the same occurs in the juxtamedullary glomeruli, more lobules and loops are formed but even here to a limited degree (fig. 5).

The large number of chicks employed in these experiments at different ages and salt concentrations, including 10 week old birds that had been given increasing concentrations of salt in their food up to as high as 20 and 25 per cent over a period of two months, enabled us to follow the sequence of changes in the glomeruli. In the early stages, associated with the ingestion of salt in excessive proportions, the glomerular capillaries widened and were apparently more tortuous. This was soon followed, usually within a few days, by (a) an increase in the size and the volume of the core and (b) the formation of glomerular loops and lobules. Both these processes were dependent on the proliferation by mitotic division of the nuclei of the core. In the formation of a loop or lobule, the core grew outward toward the capsular space as a broad or narrow, shallow or deep bulge or process that carried with it basement membrane, capillaries and visceral epithelium. This evidently differs from the mammalian glomerular loop in having a solid center with a blood vessel about the periphery. From the primary loops, secondary ones at times appeared to develop. Aside from the variations in length or breadth of the loops, there were variations in their numbers, the loops being so numerous at times as to lend to the surface a papillary appearance.

The degree to which these glomerular growth processes occurred in comparison with the controls was subject to individual variations dependent on age, rate of growth, duration of the experiment and concentration of salt employed. Except for the immediate subcapsular ones, all the glomeruli of the cortex might be enlarged, with progressive increase in size from the peripheral to the juxtamedullary ones. On the other hand, the enlargement might be confined to the juxtamedullary glomeruli only. This, however, did not necessarily represent a fixed state inasmuch as universal glomerular enlargement may have occurred at one time during the course of the experiment, but as more of the work of renal filtration was taken over by the large and enlarging juxtamedullary glomeruli, the others might have regressed or have remained stationary owing to the fact that the normal growth processes had overtaken them. By the same token the peripheral ones might have undergone later enlargement, if those more centrally had been unable to cope with the load imposed by increased intake of fluid. The enlargement of the juxtamedullary glomeruli was, however, constant.

The striking extent to which these glomerular changes occurred may readily be seen by contrast with the controls in figures 1 to 6. The huge glomeruli were often markedly looped and lobulated, but at times the capillaries pursued a fairly direct course over a greatly enlarged core. The smaller or even normal-sized peripheral glomeruli in the experimental birds showed irregular surfaces owing to the formation of many short loops as compared with the smooth-contoured, nonlooped or poorly looped surfaces of glomeruli in the same location in the controls.

The glomerular hypertrophy described served to increase the area of glomerular filtration by increasing the size of the capillary bed.

Associated with these hypertrophic changes in the glomeruli there were often two additional striking pathologic changes. The first of these consisted of increased deposition of collagen and reticulum beneath the basement membrane, particularly within and between the loops (figs. 7 and 8). In fact, the whole loop might be replaced by connective tissue, with almost total disappearance of the nuclear elements. From these fibrosed areas, coarser, more abundant reticular fibers swept through the core toward the increased reticular deposits about the hilus of the glomerulus. In sections stained with hematoxylin and eosin this fibrous material presented itself as a very pale staining, fairly spongy eosinophilic matrix. With Heidenhain's azocarmine it stained deep blue and appeared to be continuous with and forming a part of the basement membrane. With Van Gieson's stain, however, it was pink-red and sharply distinguishable from the basement membrane. When stained for reticulum, it was found to be made up of interwoven discrete or fused argyrophilic fibrils, quite distinct from the nonargyrophilic basement membrane. The second pathologic variant was the deposition of fine droplets of isotropic sudanophilic fat within the fibrosed or reticular matrix.

Two false appearances were produced as a result of the fibrosis in hypertrophied glomeruli: (1) The basement membrane of the glomerular capillaries appeared unusually thickened, and (2) the glomeruli so affected appeared avascular. It could be shown, however, by employing appropriate stains, such as Van Gieson's or those for reticulum, and by viewing the loops in different planes, that the basement membrane was at no time appreciably thickened. Likewise, it could be demonstrated, by examining thick sections, that the fibrosis of the glomerulus in no way involved the filtering surfaces of the capillaries, their number or to any extent their caliber. It may be presumed, therefore, that these productive and fatty changes were associated with undue mechanical stresses placed on the glomerular capillaries but that in themselves they interfered little with the enhanced flow of blood through, and filtration by, the glomerulus. This view is supported by the following facts:

1. These glomerular changes were most marked in younger experimental birds whose daily water consumption was progressively increasing, whereas it was not nearly as apparent in older birds with more stable, although elevated, fluid intake and with large cardiorenal reserves.

2. An occasional glomerulus in the controls of the third experiment revealed similar, though less marked, changes.

3. In the birds that were killed three, ten and twenty-eight days, respectively, after having been re-
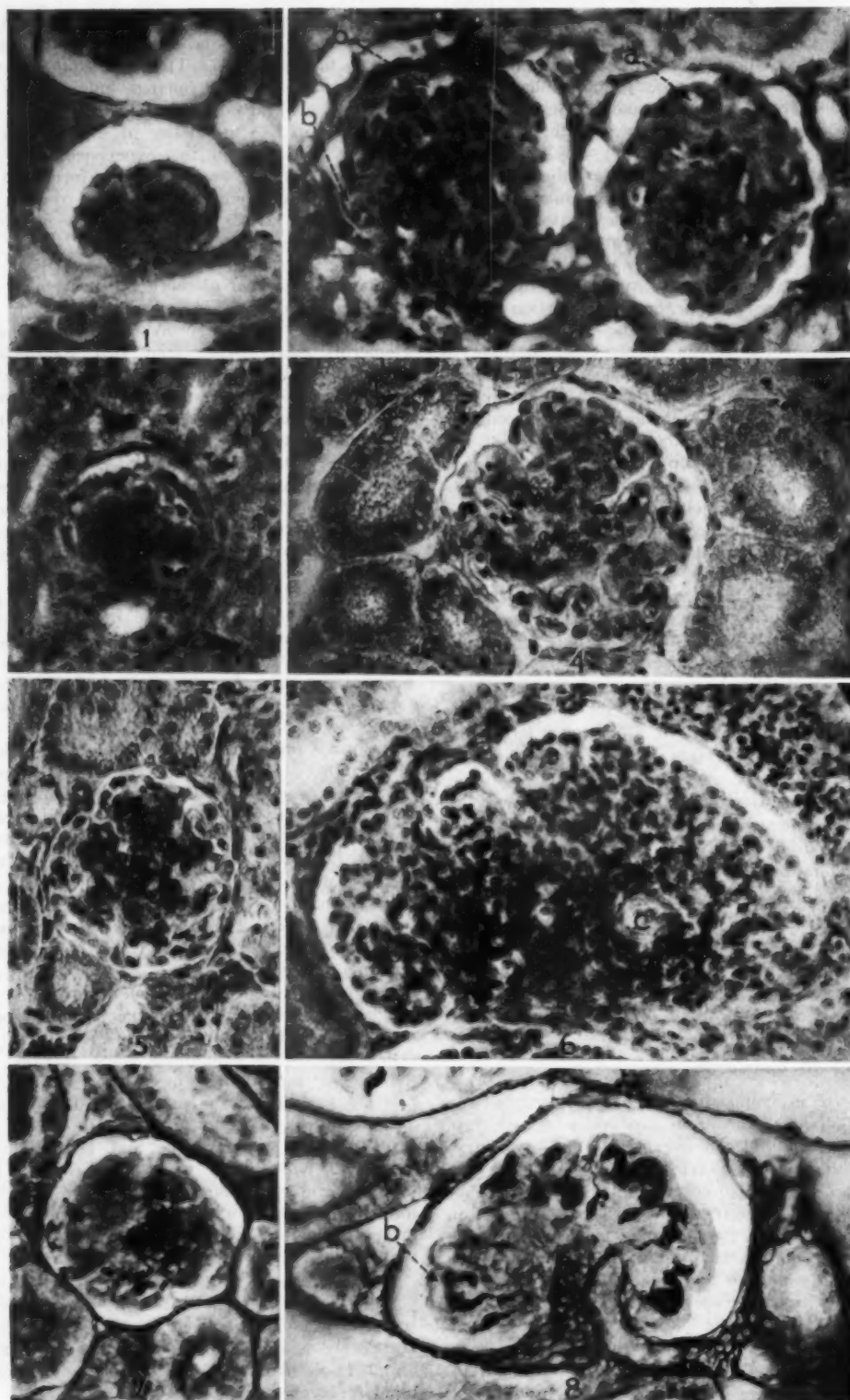
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turned to a normal feed following a three week period of maintenance on a 3 per cent salt–food mixture, the glomeruli assumed essentially normal appearances at the end of twenty-eight days. The glomerular changes described were definite in the bird killed after three days and were much less evident in that killed after ten days. This apparent reversibility of the hypertrophy and fibrosis of the glomeruli will have to be verified in a larger number of birds.

The other changes in the glomeruli can be stated briefly. The nuclei of the core varied in size and degree of vesicularity with variation in the amount of internuclear substance. The nuclei were apt to be larger and more vesicular in younger chicks or in the early stages of excessive ingestion of salt. Pyknosis and rhexis of these nuclei were also quite frequently observed. The glomerular capillaries might be relatively empty or filled with red blood cells. On rare occasions, and generally in birds that died or were killed when moribund, they contained an excessive number of granulocytes. There was never any unusual proliferation of capillary endothelium. The visceral epithelium of Bowman's capsule was often found in mitosis. These cells tended to be larger, taller and more abundant in the experimental birds. They were often filled with hyaline granules and at times with fine droplets of fat. Hence it may be assumed that in general under these experimental conditions they presented increased proliferative and degenerative changes. The capsular spaces were usually empty. Rarely they contained red blood cells. The parietal epithelium of the glomerular capsule was generally flat but at times showed hypertrophy and some proliferation. Crescents were rarely seen. Adhesions between visceral and parietal capsular surfaces occurred occasionally and consisted of epithelial strands with or without some connective tissue. There was an increased amount of connective tissue and an increase in the number of small blood vessels about the very large glomeruli. Almost as prominent, however, was an increase of small blood vessels about the large juxtamedullary glomeruli in the controls. Very large glomeruli like the one in figure 6 presented intraglomerular arteriolar branches with well defined medial coats, while their capsular space was traversed by a few delicate septums.

The renal tubular changes were slight. From a microscopic point of view, poor development or atrophy, in accord with the state of growth and nutrition of the bird, was observed. In young chicks receiving excessive amounts of salt and showing poor growth, the tubules were poorly developed, and the epithelium was lower than that in the controls. With good growth despite the excess of salt there was no essential difference in tubular development or in height of epithelium when these were compared with the controls. There was no appreciable degree of enlargement of the tubular lumens. There were three types of tubular changes which were seen with greater frequency in the experimental kidneys: (1) an increased number of casts made up of red blood cells, (2) regenerated tubular sectors made up of more basophilic, flatter, more closely crowded cells and (3) fine droplets of fat in the epithelium of some of the tubular sectors, whereas in the controls such droplets were generally absent.

The arterial branches of the kidney at no time showed any changes even remotely resembling arteriosclerosis or arteriolosclerosis. There was growth with thickening and enlargement of those in the inner third of the cortex in birds whose excessive salt intake was long continued. These, however, presented no intimal or medial changes that could be construed as arteriosclerotic. There was more abundant medial smooth muscle, as well as an increased amount of fibroelastic adventitial tissue.

In birds whose growth was impaired the nephrogenic layer showed varied degrees of retarded or arrested development, but in those whose growth was proceeding normally development of this layer was as active as in the controls. Interstitial renal edema occurred only in the presence of ascites and was often profound. In some very young experimental chicks, active intravascular hemopoiesis was observed in the subcortical venous sinusoids. The nature of the renal cysts could not be determined but they seemed to be enlarged lymphatics.

COMMENT

The toxic effect of excessive salt in birds has been known for a long time. The scanty literature dealing with investigations of this effect

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EXPLANATION OF PLATE

Fig. 1.—Glomeruli of a 15 day old control cockerel.

Fig. 2.—Glomeruli of an 18 day old cockerel which had been reared on a food mixture with a high salt content since a day after it was hatched.

Fig. 3.—Glomerulus of a 65 day old control pullet.

Fig. 4.—Glomerulus of a chick of the same sex and age as that of figure 3 but given 0.9 per cent saline solution to drink for forty-three days.

Fig. 5.—Glomerulus of a control pullet 124 days old.

Fig. 6.—Glomerulus of a chick of the same sex and age as that of figure 5 and maintained on the same feed except that 3 per cent salt was added, from the time it was 3 weeks old.

Figs. 7 and 8.—Reticulum stains of glomeruli of the same control and experimental birds as those represented in figures 3 and 4.

The marked hypertrophy of the glomeruli of the experimental birds on the right is readily apparent. Note the loops and lobules in figure 4 and the filiform character of the many loops in figure 6. Note the wide patent capillaries in figure 2 despite the marked reticulosis or fibrosis of the loops. In a the dilated capillary appears to be separated from the glomerular space by a thick membrane. This is due to an oblique plane of section. As seen in b, these capillaries reach the filtering surface without any appreciable thickening of the basement membrane. Such wide patent capillaries, sectioned in the appropriate plane, are seen in the fibrosed loops in figure 4 and particularly well in b of figure 8. C in figure 6 represents an intraglomerular arteriole.

Magnification, × 630.

aside from reports of field cases was reviewed in 1932 by Quigley and Waite.[9] The earlier investigators were concerned chiefly with the amount of sodium chloride that was toxic for the adult bird when administered in a single dose or when given in repeated doses (Zurn[10]; Collier[10]; Suffran[10]; Edwards[10]; Mitchell, Card and Carman[11]). Under these circumstances an unquenchable thirst develops and the birds generally die before the hypertonicity of the body fluids conveying the salt can be reduced to physiologic levels. The toxicity here is due to hypertonicity and profound cellular dehydration. Feeding salt, however, within limits which permit adequate dilution creates a different problem, one in which the volume of water imbibed is the principal factor. The condition differs from pure water intoxication, for here there are adequate sodium and chloride ions to retain the water in the interstitial spaces without affecting appreciably the tonicity and to a degree the acid-base balance of intracellular and extracellular fluids. It is surprising, however, how well birds can compensate for the large amounts of fluids which they take when there is an excess of salt in their food. Mitchell, Card and Carman[11] found that chickens could be reared from 9 to 21 weeks of life on rations containing as high as 8 per cent sodium chloride, with no apparent detrimental effects. The water consumption was not determined. Smith[12] reared chicks from a day old to 8 weeks on varying levels of salt from 0 to 4.5 per cent. Food and water consumption were determined by lots for the whole period of observation. There was a marked increase in water consumption from 8.08 quarts per bird for the entire period in the lot with no salt to 13.5 quarts in the lot whose feed was 4.5 per cent salt. The food consumption dropped, however, in the same categories from 2,622 Gm. to 2,467 Gm. The mortality was 5 per cent in the lot without salt and 20 per cent in the lot receiving 4.5 per cent salt. He described the puffy appearance of the birds due to edema and the dyspnea, which in those on levels of salts higher than 2 per cent he ascribed to acidosis.

Quigley and Waite[9] repeated these experiments with newly hatched chicks, varying the salt content of the feed from 3 to 15 per cent. The mortality varied from about 7 per cent on 1 per cent salt to 77 per cent on 15 per cent salt. Growth curves revealed decreasing levels with increased salt. Although the water consumption was not determined, they observed the marked thirst and watery diarrhea. They recorded the appearance of bloat within twenty hours on the most heavily salted rations, within forty-eight hours on those with 10 per cent and within six days on rations with 3, 5 and 8 per cent salt, respectively.

In all these earlier experiments, histologic studies apparently were not performed. Recently, however, Selye[13] described a syndrome of nephrosclerosis or Bright's disease in chicks treated with desoxycorticosterone alone, with 0.9 per cent saline solution as drinking water alone or with combinations of the steroid and lower concentrations of saline solution. The diagnosis was based on the presence of glomerular fibrosis, crescents and tubular degeneration. He divided this form of Bright's disease in 19 day old chicks given 0.9 per cent saline solution to drink into a wet stage with edema, developing in the first ten days of the experiment, and a dry stage with cardiac hypertrophy, occurring in the second ten days. He has ascribed this to the greater sensitivity of fowl to sodium chloride in contrast with the comparative resistance of mammals to overdosage with salt.

From our own observations the reasons for this greater sensitivity of the fowl to sodium chloride appear to be at least three in number: 1. The bird appears to be less discriminating in its taste. It will continue to satiate its hunger and thirst despite the salted character of the food or of the water it is given, to and beyond its limit of tolerance. The mammal is more apt to control its hunger and thirst and will if necessary completely avoid salted food or water. 2. The renal glomerulus of the bird offers a considerably smaller area for filtration as compared with the looped and tufted glomerulus of the mammal. 3. The normally lower level of the total plasma proteins in the bird renders maintenance of intravascular and extravascular osmotic equilibrium difficult. It seems likely, too, that in birds the extravascular accumulations of fluid, whether in subcutaneous spaces or in serous cavities, are accompanied by outpouring of a large amount of plasma proteins. This was true of the "exudate" described by Dam and Glavind in their vitamin E–deficient chicks and is true of the chicks

9. Quigley, G. D., and Waite, R. H.: Bulletin 340, University of Maryland Agricultural Experiment Station, College Park, Md., December 1932.

10. Cited by Quigley and Waite.[9]

11. Mitchell, H. H.; Card, L. E., and Carman, G. G.: Bulletin 279, University of Illinois Agricultural Experiment Station, 1926.

12. Smith, H. J.; Toxicity of Salt for Chicks, Circular Letter, Purina Mills, St. Louis, Mo., Sept. 24, 1929.

13. Selye, H., and Stone, H.: Proc. Soc. Exper. Biol. & Med. 52:190, 1943. Selye, H.: J. Am. Vet. M. A. 103:140, 1943.

with high salt intake studied by us. This tends to deplete the plasma proteins more rapidly, creating a vicious circle with increasing edema.

It is basically the huge consumption of water by birds receiving excessive salt that accounts for the edema and the cardiorenal changes. The fact that the edema appears in 3 week old chicks within twelve hours after they are given a food mixture containing 3 per cent salt is certainly against the view that it is on a glomerulonephritic basis. The fact, too, that even in the presence of obvious edema the accumulations of fluid rapidly disappear when the bird is returned to a normal feed, as we observed on various occasions, is likewise against such a view. The presence of edema and the progression or the spontaneous regression of the edema, as well as the mortality, were dependent on the age, the sex, the caloric intake and the cardiorenal compensatory mechanisms of the bird, in addition to the factor of the actual concentration of salt employed. Associated with age was the factor of cardiorenal reserve. In newly hatched chicks edema readily developed at a level of salt which older birds were more able to tolerate. Ten week old birds could withstand an amount of salt which 3 week old birds could not. This seemed to be correlated with the percentage relation of the amount of water consumed per day to the body weight. A value of more than 50 per cent of body weight could be regarded as exceeding the bird's tolerance. While this was readily reached in newly hatched or slightly older chicks, much higher concentrations of salt were required before it could be reached in older birds. To compensate for the increased intakes of salt and water, the food intake had to be adequate and the heart and the kidneys had to function at higher planes to eliminate the excess of salt and of water. Accordingly, the heart often was enlarged. In the kidneys a larger area for glomerular filtration was afforded by the hypertrophy of the glomerulus and the formation of lobules and loops. As has already been pointed out, the fibrosis is regarded as secondary to mechanical stresses placed on the hypertrophied glomerulus and is in no way associated with arterial or arteriolar sclerotic changes or with

glomerulitis as we understand it in mammalian pathology. The increased glomerular capillary bed remained patent with no endothelial hypertrophy or hyperplasia and with no frank acute exudative inflammatory changes. The rare crescents and the occasional capsular adhesions can be regarded as irritative phenomena which interfered but little with renal function.

Our results must be interpreted with the knowledge that the experiments were carried out under semitropical conditions and that dry food was employed. The water consumption would probably be different in temperate climates and at different seasons, with the associated variation in the insensible loss of water from lungs and skin. Accordingly, the pathologic changes produced might be somewhat different, but the basic pattern would be the same.

CONCLUSIONS

The toxicity of sodium chloride for young chicks under the field and experimental conditions detailed here is to be ascribed to (1) the indiscriminate feeding habits of the fowl, (2) the morphologically different type of renal glomerulus in the fowl as compared with the mammal, which limits the effective area of filtration, and (3) the normally low total plasma proteins and the blood status represented in low hematocrit readings. The basis for the pathologic changes associated with the ingestion of excessive salt is the accompanying huge consumption of water. In view of the fact that birds are limited in their capacity to deal with these large quantities of water in part at least by the aforementioned factors, the early appearance, the persistence and the progression of the edema are readily understandable. The earliest morphologic compensatory mechanism that is brought into play in order to aid in the elimination of the excess fluid is glomerular hypertrophy with associated new formation of loops and lobules. An extension of this process and cardiac hypertrophy are in the main the two anatomic factors concerned in determining the survival of the bird and its complete freedom from edema despite the increased consumption of water.

STRUCTURAL CHANGES IN EARLY FILARIASIS

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The literature on the morphologic changes in filariasis is limited. Hartz[1] recently described 3 cases in which biopsy revealed typical changes in the lumens and the walls of the lymphatics, in the lymph nodes and in the connective tissue. The histologic reaction consisted chiefly of an epithelioid cell endolymphangitis and perilymphangitis. Michael[2] described a proliferation of filarial granulation tissue which he considered almost pathognomonic of the disease. He further stated that the reaction becomes distinctly more specific in appearance as degeneration of worms occurs. Pasternack,[3] in a recent report, described a case in which biopsy revealed an epithelioid cell granulomatous endolymphangitis. Belding[4] wrote that there is marked swelling of the endothelium of the vessels with exudation into the lumens and eventual production of a caseating focus. O'Connor and Hulse[5] mentioned that the adult worms are found chiefly in the cortical sinuses or the afferent lymphatics of the lymph node. Our report is given to indicate that specific histologic changes are caused by filariasis.

We have had an opportunity to study a series of patients from units which had been stationed. for thirteen to thirty months in various islands of the Souht Pacific, where filariasis is known to be endemic. In addition, we have reviewed a series of lymph nodes and lymphatic vessels from patients suspected of having filariasis for whom biopsies were made at other Army medical installations. The present report is based on a detailed study of 30 cases. In the entire series the peripheral blood revealed no evidence of microfilarias. Thick and thin smears and Knott concentration methods were utilized. Hematologic examination revealed nothing characteristic. In 60 per cent of the cases there was eosinophilia, ranging from 6 to 11 per cent. In the remaining 40 per cent the eosinophilic

cell count varied from 2 to 4 per cent. The red and white blood cell counts were within average limits. Smears of the peripheral blood revealed no hematologic aberrations. Gram stains of all lesions did not reveal streptococci or other bacteria. Material from 10 of our filarial lesions was cultured. No organisms were found. Sixty per cent of the patients were seen during their first attack. The remaining 40 per cent gave a history of two or more relapses following the initial attack. Constitutional symptoms were not present in any of the patients. A small number of patients gave a typical history of painful red spots in either the upper or the lower extremities, followed by swelling and tenderness of the regional lymph nodes, followed in turn by a centrifugal or a retrograde type of lymphangitis. The vast majority of the patients had their attention first directed to their illness by painful enlarged superficial lymph nodes or by brawny edematous lymphangitis or acute epididymofuniculitis. In these patients no typical sequence of events could be discerned. Forty per cent of the patients noted primary involvement of the genitals or of the lower extremities. Thirty per cent revealed primary involvement of the upper extremities. In the remaining 30 per cent there was coincident involvement of both the upper and the lower extremities.

Biopsies of lymph nodes or segments of lymphatic vessels were undertaken in all cases. In 20 per cent positive evidence of filariasis was revealed by demonstration of the worms. In the remaining 80 per cent, the worms were not found, but the histologic picture was almost identical with that found in biopsy specimens in which the worms were shown. Michael gives two explanations for the absence of worms in these biopsy specimens: first, that the reaction is allergic, due to the presence of foci of worms elsewhere; second, that worms preexisting in these areas may have been destroyed. We shall furnish one further bit of evidence toward the acceptance of the allergic nature of the disease. All tissue was fixed in solution of formaldehyde U. S. P. (1:10). Sections were serially cut at 3 to 5 microns, so that the entire biopsy speci-

1. Hartz, P. H.: Am. J. Clin. Path. **14**:34, 1944.
2. Michael, P.: U. S. Nav. M. Bull. **42**:1059, 1944.
3. Pasternack, J. G.: Arch. Path. **35**:414, 1943.
4. Belding, D. L.: Textbook of Clinical Parasitology, New York, D. Appleton-Century Company, Inc., 1942.
5. O'Connor, F. W., and Hulse, C. R.: Puerto Rico J. Pub. Health & Trop. Med. **11**:167, 1935.

men was examined. Hematoxylin-eosin and Giemsa stains were utilized.

Fifteen patients who had been stationed in the Samoan group of islands for a period varying from fourteen to twenty months were admitted to an Army General Hospital with a clinical diagnosis of filariasis. All of them at the time of admission had enlargement of peripheral lymph nodes. They first complained of tenderness and swelling in these regions ten to fourteen days prior to admission. Biopsies were made of lymph nodes of each patient. Sixty per cent of the biopsy specimens were obtained from either the right or the left inguinal region, 20 per cent from the right axilla and 20 per cent from the left epitrochlear region. Adult worms were found in only 2 biopsy specimens. These worms were present in the afferent lymphatics. They resembled Wuchereria bancrofti. Mitoses were seen in the intestinal epithelium, and apparently the worms were alive at the time of fixation. The characteristic double-contoured uterus was visible. The cuticle took an eosin stain, appearing clear pink throughout. The embryonic microfilarias stained as deeply basophilic round granules. The histologic pictures in these nodes and in the remaining 13 biopsy specimens appeared almost identical. The changes consisted chiefly of extensive hyperplasia of the primary and secondary lymph follicles. The germinal centers revealed small numbers of mitotic figures. There was a striking infiltration of the entire node with eosinophilic leukocytes. The lymphatic channels contained a thin pink-staining albuminoid fluid. This has been previously described as "fluid lakes." A granulomatous reaction was not evident at this stage. The picture in each node was indicative of an acute allergic reaction.

The possible systemic nature of this allergic phase is demonstrated in the following 2 cases. The first patient was a 27 year old white man, who was admitted complaining of swelling, pain and tenderness in the region of the right epididymis of three days' duration. The second patient, a 22 year old white man, complained of swelling, tenderness and pain in the region of the medial surface of the upper third of the right arm. This was their first clinical attack. Each of these patients had been in known filarial regions for a period of fifteen months. On examination the first patient presented epididymofuniculitis on the right side. In addition, there was enlargement of the right epitrochlear lymph node, which had developed thirty-six hours prior to admission. The second patient presented in addition to the lymphangitis of the right arm a painful

swelling of the left inguinal node, which had developed while he was under observation in the hospital. Biopsies were made in both cases. In the first case, areolar tissue containing lymphatics and vessels was removed from the right epitrochlear region. In the second case, in addition to a segment of the inflamed lymphatic channel of the upper third of the right arm, the left inguinal node was removed for study. Histologic examination of the lymphatic vessel from the right arm revealed the lumen to be dilated and filled with a necrotic tissue. There was slight scalloping of the endothelium. The wall of the lymphatic channel was thickened, edematous and densely infiltrated with eosinophilic leukocytes. There was, in addition, moderate infiltration with small numbers of epithelioid cells, each having a clear vesicular cytoplasm with a bandlike nucleus, fibroblasts, lymphocytes and an occasional plasma cell. Multinucleated foreign body giant cells similar to those in tuberculosis were observed at the periphery of the wall. No neutrophilic polymorphonuclear leukocytes were visible. Histologic examination of the areolar tissue around the right epididymus revealed a dilated lymphatic vessel which contained an adult filaria. The wall was densely infiltrated with eosinophilic leukocytes, lymphocytes, plasma cells and an occasional multinuclear giant cell. Interspersed throughout were small numbers of epithelioid cells. Immediately surrounding the lymphatic wall was a dense collection of pyknotic eosinophils and epithelioid cells. Close to the periphery of the hyperplastic lymphatic vessel was a characteristic eosinophilic tubercle, consisting of a central area of necrosis surrounded by accumulations of eosinophils and epithelioid cells. The lymphatic tissue from the left epitrochlear region and the lymph node from the right inguinal region, from the first and the second patient, respectively, presented a characteristic allergic reaction. The lymph node revealed hyperplasia of the lymph follicles, "fluid lakes" and a moderate eosinophilic infiltration throughout. The lymphatic vessel was dilated and had an edematous wall with moderate numbers of eosinophils ramifying throughout. The lumen was filled with a pink-staining albuminoid material. Neither microfilarias nor macrofilarias were found in these sections. These cases are of interest so far as they appear to be indications of a possible systemic allergic reaction.

In 13 cases that came to our attention histologic changes were revealed which we consider to be a specific proliferative granulation tissue reaction caused by filariasis. Five of these

cases were studied clinically by us. In the remaining 8 cases biopsies were made in other Army Medical units and sent to us for histologic study. In 3 of these cases filarial foci were found. In the remaining cases, the histologic picture was identical, but no parasite was visible. We present 3 of these cases.

REPORT OF CASES

CASE 1.—A white soldier 29 years of age was admitted with a history of living for twenty-six months in an area in which filariasis was endemic. He had had three attacks of clinical filariasis, all involving the right upper extremity. In the last four months he had noticed progressive enlargement of the right axillary lymph node, with no tenderness or pain. Constitutional symptoms were not present. A biopsy of the axillary node was made. It revealed a typical proliferative granulomatous change. There were large collections of epithelioid cells throughout the lymph node. These were aggregated, forming small tubercles. In many areas they were associated with multinucleated Langhans giant cells. The individual epithelioid cell was irregular in shape and had a bandlike nucleus, a distinct nuclear membrane and a clear reticular cytoplasm. In one afferent vessel an adult filaria (W. bancrofti) undergoing degenerative change was demonstrated. Within its uterus were many deeply basophilic microfilarias which had undergone pyknotic changes. The endothelial lining of the lymphatic vessel in which these worms were present was definitely indented into the lumen, producing a striking effect of scalloping. A deep zone of necrosis was seen immediately around the worn segments. This area was surrounded by large numbers of epithelioid cells, lymphocytes, plasma cells and young fibroblasts. A chracteristic feature was the proliferative reaction observed in the lumens of the afferent and efferent lymphatics. Large numbers of epithelioid cells, with an occasional lymphocyte and plasma cell, were seen filling the lumens. These cells were accompanied by multinucleated giant cells, and in a number of areas they had actually produced occlusion of the vessels. A similar granulomatous reaction was seen immediately surrounding the lymphatic vessels. One vessel had thin strands of eosinophilic collagenous connective tissue ramifying into the lumen between the cells of the infiltrate. In the periglandular connective tissue a similar proliferative reaction was visible.

CASE 2.—A 25 year old white man had been for a period of fifteen months in areas in which filariasis was endemic. There was a history of three previous relapses, all involving the left upper extremity. For the past three months he had noticed progressive painless enlargement of the right epitrochlear lymph node. A biopsy was made. It revealed small epithelioid cell tubercles with multinucleated foreign body giant cells. The lymph follicles were moderately enlarged. The lymph sinuses were infiltrated with epithelioid cells, mononuclear histiocytes and lymphocytes. Present in an afferent lymphatic vessel was a dead adult worm. The endothelial lining was indented into the lumen. Immediately surrounding and involving the wall of the vessel was a central zone of necrosis, surrounded in turn by large accumulations of epithelioid cells, lymphocytes, plasma cells and small numbers of fibroblasts. Multinucleated Langhans giant cells were seen throughout. At the periphery was a thin layer of hyalinized fibrous connective tissue, apparently attempting to localize the lesion. The intermediate sinuses and the afferent and efferent lymphatic channels revealed an endolymphatic and a perilymphatic proliferative granulomatous reaction. Small epithelioid cell tubercles were present in the periglandular connective tissue. Eosinophilic and neutrophilic leukocytes were not visible.

CASE 3.—A 23 year old white man had been for a period of seventeen months in an area in which filariasis was endemic. He was a member of the same unit as the previous 2 patients. He complained of redness, soreness and swelling of the left forearm of three days' duration. There was no history of previous attacks. Examination revealed an induration measuring 10 cm. in length on the medial surface of the left forearm, with superficial redness and tenderness to pressure. An enlarged lymph node was palpated deep in the left axilla. The remainder of the examination revealed no abnormality. Roentgen examination of the chest gave negative results. Histologic examination of the axillary lymph node revealed partial preservation of the nodal structure. There was extensive hyperplasia of the lymph follicles. Scattered throughout the node were tiny aggregations of epithelioid cells. These were most prominent in the germinal centers and intermediate sinuses. A number of these cells had fused to produce typical Langhans giant cells. The individual epithelioid cell was irregular in shape and had a clear pale reticular cytoplasm, a vesicular nucleus and a distinct nuclear membrane. They could easily be distinguished from the surrounding lymphoid tissue. These lesions varied from submiliary collections of epithelioid cells to large conglomerate tuberculoid foci. In one area each lesion revealed an intense eosin-staining caseous-necrotic center with two or more giant cells around the outer margin of the caseous material. A proliferation of epithelioid cells, concentrically arranged at the periphery, accompanied this caseation and necrosis. The peripheral zone consisted of lymphocytes, plasma cells and young spindle-shaped fibroblasts. Eosinophilic and neutrophilic leukocytes were not observed. In the afferent and efferent lymphatic channels a proliferative granulomatous reaction was visible, similar to that seen in the preceding 2 cases. Serial sections revealed no evidence of adult worms or of microfilarias. Ziehl-Neelsen stains did not reveal tubercle bacilli.

The changes observed in these cases appear to represent the proliferative stage in filariasis, manifested by a granulomatous reaction within the lumens of the lymphatic vessels, involving the walls of the latter and the entire structure of the lymph node. In those cases in which the worms or filarial foci were not demonstrated, it may well be that there had been disintegration of previously existing worms.

COMMENT

A histologic study of specimens of lymphatic tissues from 30 patients with clinical filariasis is presented. In 6 cases a positive diagnosis of filariasis could be made by the demonstration of microfilarias or an adult filaria in the lymphatic vessels or the lymph nodes. The histologic pictures in these specimens and in those in which the worm was not demonstrated were almost identical.
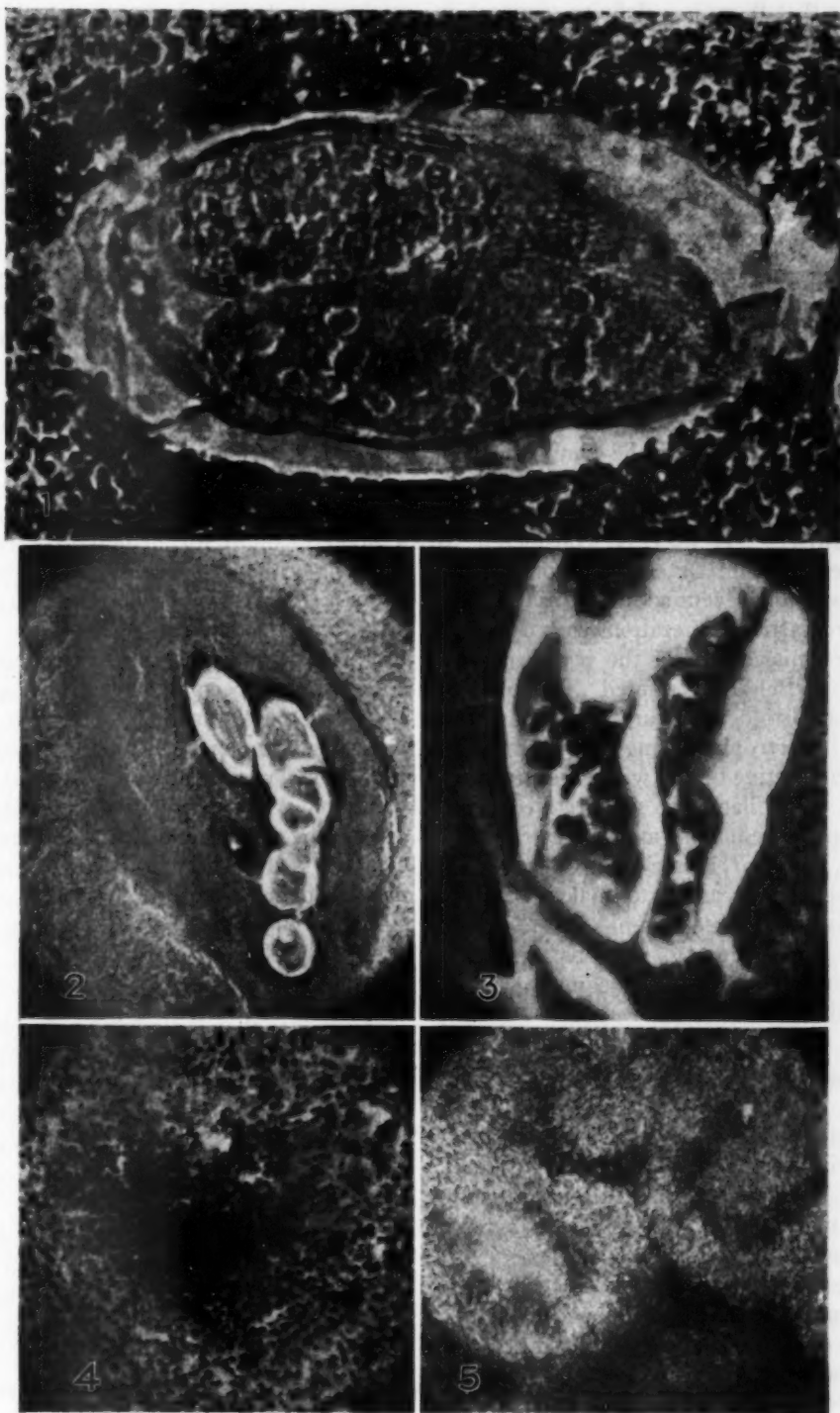
Fig. 1.—Adult worm, degenerating, in lymphatic vessel. Note the extensive collection of pyknotic eosinophils infiltrating the vessel wall. Hematoxylin and eosin; × 400.

Fig. 2.—Degenerating worm foci in an axillary lymph node. Note the scalloping of the endothelial lining and the characteristic granulomatous tissue reaction around the worm. Hematoxylin and eosin; × 200.

Fig. 3.—Tangential section showing larval forms coiled with their eosin-staining sheaths in the midsegment of the uterus. Hematoxylin and eosin; × 300.

Fig. 4.—Eosinophilic tuberculoid focus in wall of a lymphatic vessel of areolar tissue of the right epididymis. Note the central necrosis surrounded by an accumulation of eosinophils, epithelioid cells and round cells. Hematoxylin and eosin; × 200.

Fig. 5.—Axillary lymph node with necrocaseating conglomerate tuberculoid foci; the worms have completely disintegrated. Hematoxylin and eosin; × 200.

We believe that there is a definite sequence in the pathologic changes induced in filariasis. It appears that the first reaction is essentially allergic in nature and that this reaction may, on occasion, be systemic and due to the presence of parasites in one of the lymphatic regions of the body. The allergic reaction is characterized by eosinophilia, edema and hyperplasia. We believe further, with Michael, that many enlargements of lymph nodes and swellings of lymphatic vessels are therefore reversible. We prefer to call this stage the acute phase of filariasis. In the lymphatic tissue wherein the parasite resides a typical granulation tissue reaction may eventually be induced. These changes occur within the lumens and the walls of the lymphatic vessels and in the lymph nodes. We subscribe to the term "epithelioid cell endolymphangitis and perilymphangitis."[1] It may be considered to designate the subacute or early chronic stage of the disease. The eventual result may be an extensive fibroblastic proliferation, with complete fibrous tissue replacement of the involved lymphatic tissue. Belding[4] quoted Suarez who considered the histologic picture in elephantiasis to consist of hypertrophic changes of dermal and hypodermal connective tissue, with extensive proliferation of the collagenous connective tissue. In some lesions there may be an associated mononuclear and polymorphonuclear cell reaction. We have not had occasion to make a biopsy of tissue from a true elephantoid area. However, in the granulomatous regions described there is distinct indication of beginning fibroblastic growth.

A problem which remains still to be answered is that of the high percentage of asymptomatic patients. It is a well known fact that there are many patients with freely circulating microfilarias in their peripheral blood who never have lymphangitis or elephantiasis. Perhaps, there is in these a natural host resistance, attributable to high immunity of tissues, in which the reticuloendothelial system plays an important part. A current controversy on the continent is whether filariasis and elephantiasis are manifestations of the same disease. In recent contact with French colonial medical officers, we have become acquainted with their views. In a recent edition of the "Clinique chirurgicale," Botreau-Roussel[6] summarized these views. It is claimed that the lymphangitis in filariasis is secondary to the filarial worm but that a streptococcus is the

etiologic factor in elephantiasis. He mentioned that this streptococcus is distinct and has been isolated at the Pasteur Institute in Paris. Geographically, filariasis and elephantiasis do not necessarily occur together. It is claimed by Roussel that elephantiasis is contagious, while filariasis is not. Grace[7] considered that the recurrent attacks of lymphangitis and elephantiasis were due to streptococcic invasion and that the filarial infection in itself bears little or no relationship to these clinical manifestations. In Michael's series, only 1 of 100 cultures from filarial lesions revealed bacterial complications. These were due to presence of Staphylococcus albus, which was considered a cutaneous contaminant. Belding summarized these conflicting views, stating that the pathologic changes in the lymphatic vessels produced by filariasis furnish a good medium for hemolytic streptococci and that superimposed infection favors fibroblastic growth. We have been unable to demonstrate streptococci by culture or by Gram stain in any of the lesions, yet a distinct reticuloendothelial response had been induced. We are in accord with those who favor the concept that filarial infection is independently responsible for recurrent lymphangitis and an eventual elephantoid reaction. We believe that the soldier group in our series are in good physical condition, that cleanliness has been maintained and that the chances of secondary streptococcic or other pyogenic infection have been reduced to a minimum.

SUMMARY

It appears that the adult filaria has the ability to produce a distinct reticuloendothelial response. This response varies with the viability of the parasite. In those cases in which a distinct endothelial reaction is induced one must rule out tuberculosis, syphilis and the other chronic granulomas.

There appear to be three stages in the genesis of filariasis: first, the acute stage, manifested by a typical allergic reaction, which may be local or systemic; second, the subacute or early chronic stage, characterized by the development of a proliferative granulation tissue and occurring in lymphatic tissues harboring the parasites; third, the late chronic stage of the disease, typified by a nonspecific fibrous tissue overgrowth. As in all classifications, no distinct delineation is possible. One stage merges imperceptibly into the other.

6. Botreau-Roussel: Clinique chirurgicale, Paris, Masson & Cie, 1938.

7. Grace, A. W.: J. A. M. A. 123:462, 1943.

CHANGES IN THE MUSCULATURE OF THE GASTROINTESTINAL TRACT AND IN THE MYOCARDIUM IN PROGRESSIVE MUSCULAR DYSTROPHY

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Four cases of progressive muscular dystrophy have been investigated at necropsy in Goldwater Memorial Hospital within the past five years. It is the purpose of this paper to review these cases with particular reference to lesions in the myocardium together with certain changes in the smooth muscle tissues of the gastrointestinal tract. One of these cases has already been recorded by Rosenthal and Tobias [1] to emphasize the occurrence of multiple perforations of the stomach of unknown cause occurring in association with acute phlegmonous gastritis. Although Erb [2] in 1884 separated the muscle dystrophies from the muscle atrophies of spinal origin, the cause of progressive muscular dystrophy is still obscure. For many years it was universally regarded as an intrinsic disease of muscle. In more recent times, however, Kuré [3] and Bramwell [4] have advanced the view that it is a disorder of the autonomic nervous system. In 1923 Globus [5] recorded a case in which the myocardium was diseased and reviewed 10 similar cases which he collected from the literature. Among the myocardial changes are diffuse scarring and deposits of fat in the scar tissue. Since then Schliephake,[6] and Berblinger and Dukens [7] have described a so-called cardiointestinal syndrome associated with progressive muscular dystrophy. This syndrome consists of vomiting, generalized abdominal tenderness, abdominal pain, diarrhea, tachycardia and signs of cardiac failure. In the 1 case of their 4 which was investigated at necropsy the description of the changes in the gastrointestinal tract is limited to atony of the stomach (sic) with erosion of the mucosa and bloody gastric contents. The terminal illness of the patient whose case was described by Globus began with vomiting.

Bunting,[8] in 1908, was among the earliest to note anatomic lesions of the smooth muscle tissue of the stomach, stating: "In numerous fasciculi there was numerical atrophy of fibres and the fasciculi were invaded by connective tissue as was clearly shown by a Mallory stain. Some of the remaining fibres were vacuolated and degenerated in appearance and others appeared hypertrophied." Potter,[9] in the following year, mentioned another instance of myocardial fibrosis in progressive muscular dystrophy. Peritz [10] quoted Leri to the effect that there are atrophy and loss of muscle in the intestine but gave no reference to the original paper. Kuré [11] recorded roentgenologic changes in the esophagus similar to those which he had described in the same situation in scleroderma, namely, delay in the passage of a barium sulfate meal. Yoshida [12] described changes in the striated muscle of the tongue and in the upper part of the esophagus in a patient who also presented lesions in the myocardium. Hurwitz [13] reviewed the records of 36 patients with primary myopathies but mentioned only one report of a necropsy, in which, however, there was no reference to changes in the heart or the gastrointestinal tract. Among the terminal illnesses noted in his series were those of 1 patient who was markedly dyspneic, 1 with signs of cardiac failure and 1 with signs of gastroenteritis. A patient who was living at the time that his report was made was said to be suffering from difficulty in swallowing and ventricular extrasystole. Goodhart,[14] reviewing 4 cases which

1. Rosenthal, J., and Tobias, M. J.: Am. J. Surg. 59:117, 1943.

2. Erb, W.: Arch. f. klin. Med. 34:464, 1884.

3. Kuré, K.: Lancet 1:441, 1928.

4. Bramwell, E.: Lancet 2:1103, 1925.

5. Globus, J. H.: Arch. Neurol. & Psychiat. 9:59, 1923.

6. Schliephake, E.: Ztschr. f. Kinderh. 47:85, 1929.

7. Berblinger, W., and Dukens, J.: Ztschr. f. Kinderh. 47:1, 1929.

8. Bunting, C. H.: Am. J. M. Sc. 135:244, 1908.

9. Potter, F. C.: New York M. J. 90:398, 1909.

10. Peritz, G., in Kraus, F., and Brugsch, T.: Spezielle Pathologie und Therapie, Vienna, Urban & Schwarzenberg, 1924, vol. 10, pt. 1, p. 931.

11. Kuré, K.; Yamagata, K.; Tsukada, S., and Hiyoski, J.: Klin. Wchnschr. 15:516, 1936.

12. Yoshida, T.: Jap. J. M. Sc., V, Path. 5:63, 1940.

13. Hurwitz, S.: Arch. Neurol. & Psychiat. 36:1294, 1936.

14. Goodhart, S. P.: J. Mt. Sinai Hosp. 9:514, 1942.

had been investigated at necropsy, mentioned fatty degeneration of the heart in a child of 14 and, in another patient, paralytic ileus developing seventy-two hours after a surgical operation. No histologic descriptions of the cardiac changes or of the changes in the gastrointestinal tract were included in his report. In the recent studies of Shank and others [15] it is stated that no symptoms referable to the gastrointestinal tract were observed clinically in a series of 40 patients with progressive muscular dystrophy. Even from this short review of the literature it is apparent that the changes in the smooth muscle tissue of the gastrointestinal tract or elsewhere have not received adequate attention.

REPORT OF CASES

CASE 1.—E. L., a 20 year old white man, was admitted to the hospital Sept. 11, 1942 and died Dec. 25, 1943. The earliest symptoms of progressive muscular dystrophy were noted by the parents when the patient was 4 years old. There is no history of the disease in the family and the only sister is a normal child. Until the age of 9 the patient managed to get about, despite marked wasting of the lower extremities. That year he entered the Children's Hospital School in Baltimore, where a diagnosis of progressive muscular dystrophy was made. He received acetylcholine, without improvement. The following year he became bedridden but remained at home until August 1941, when he entered the Hospital of the Rockefeller Institute. Early in 1941 he received aminoacetic acid in unknown amounts for a period of three months. In December of 1941 an enlarged heart and signs of cardiac decompensation were noted. Digitalis was administered and the condition improved greatly. At this time the small excursion of the left ventricle was recorded in a kymogram. In April 1942 he showed signs of decompensation and was given maintenance doses of digitalis. The digitalization was continued until his death. He was given liver parenterally, as well as vitamins B and C, without benefit. The patient remained at the Rockefeller Institute from August 1941 until his transfer to this hospital in September 1942.

On admission there was wasting of all the musculature. There were no fibrillations. The rate of the radial pulse equaled that of the ventricle and was 90 beats per minute. The blood pressure was 120 systolic and 95 diastolic. There was facial asymmetry due to flattening of the nasolabial fold on the right side, and there was marked wasting of the skeletal musculature, together with contracture of the tendons of the lower extremities. The feet were held in the position of equinovarus. There was abnormal mobility of the shoulder joint and of the small joints of the hands. All reflexes were absent except the cremasteric ones, which were normal. A luxuriant growth of hair was noted over the extremities and the abdomen. The heart was not enlarged to percussion. The veins of the neck were not distended. The lungs were clear. The chest was flattened in the anteroposterior diameter. The liver, the spleen and the kidneys were not palpable. Five months after admission, gallop rhythm was heard at the apex. Serial electro-

cardiograms revealed right axis deviation and sinal tachycardia. The rate never rose above 110. The gallop rhythm disappeared, and for several months the patient was in fairly good condition. Nov. 27, 1943 he complained of a feeling of pressure in the chest. There were rhonchi and rales, and the temperature ranged between 100 and 102 F. His condition was unchanged for the next month; then he began to have difficulty in swallowing and later in coughing up sputum. He was placed in a respirator, and the secretions in the bronchi were aspirated whenever necessary. Death occurred on Dec. 25, 1943.

Laboratory Data. — The basal metabolism rate on many occasions ranged between —15 and —10 per cent of normal. The circulation times on Oct. 6, 1942 were slightly prolonged. The vital capacity on Nov. 25, 1942 was 3.7 liters.

The blood sugar during fasting was 85 mg. per hundred cubic centimeters. The blood urea nitrogen was 11 mg. per hundred cubic centimeters.

The urine concentrated to 1.032, and routine examinations gave normal results.

Roentgenograms of bones showed a moderate degree of decalcification and considerable underdevelopment. The sella turcica appeared to be normal. There was no calcification in the falx. The heart was enlarged in all diameters. The esophagus was slightly displaced by the dilated right auricle. Two weeks before he died, areas of consolidation were seen in various parts of the lungs.

Treatment at this hospital was directed only to counteracting heart failure and included the use of digitalis.

Necropsy (nine hours after death).—Abundant hair was seen over the arms, the abdominal wall and the lower extremities. The legs were in a froglike position. There were contracture of the hamstrings and bilateral pes cavus. Abnormal mobility of hips, shoulders and wrists was apparent, and the interphalangeal joints could be hyperextended.

Wasting of all muscles of the extremities and the trunk was marked. The pectoral muscles were pale, thin and streaked with yellowish tissue corresponding to fat. The iliopsoas muscles were similar. The intercostal muscles were pale but were of normal thickness. The gastrocnemius muscle was entirely replaced by fat.

The chest was flat in the anteroposterior diameter, and the lower ribs flared. The cortex of the ribs was not diminished. The precordial area was large. There was no free fluid in the chest. A small remnant of thymus was attached to the right lung.

The lungs contained a moderate amount of fluid. There was a small area of consolidation in the lower lobe of the left lung. It was yellow and surrounded by a hemorrhagic zone.

About 10 cc. of clear fluid was found in the pericardial sac. The epicardium over the surfaces of both ventricles showed several white opaque patches. The epicardial fat was abundant. The heart was hypertrophied, and all the chambers were greatly dilated. It weighed 420 Gm. The endocardium lining both ventricles was uniformly thickened and opaque. There were no thrombi in the heart. The valves were thin and delicate. The myocardium was flabby, tan colored and streaked with fibrous tissue. Beneath the endocardium of the papillary muscles were several small ecchymoses. In an area about 5 cm. in diameter over the posterior wall of the left ventricle, the myocardium bulged, and section showed almost complete replacement of the muscle by scar tissue. The left ventricle varied in thickness from 1.2 cm. near the aortic valve

15. Shank, R. E.; Gilder, H., and Hoagland, C. L.: Arch. Neurol. & Psychiat. **52**:431, 1944.

to 0.3 cm. near the apex. The right ventricle measured 0.6 cm. in its thickest portion and appeared to be hypertrophied throughout. There was relatively more normal muscle tissue in the right than in the left ventricle. The coronary arteries were entirely free from sclerotic plaques. The aorta was hypoplastic, and no sclerosis was evident.

The abdominal panniculus measured 3 cm. in thickness. The rectus abdominis muscle was so thin that it was scarcely discernible between the fasciae. The dome of the diaphragm was at the fourth interspace on the right and at the fifth on the left. The muscle was thin and pale tan.

The tongue was large, and yellow streaks of fat separated the pale muscle. The esophagus, the stomach and the small intestine were of normal caliber, and the mucosa was well preserved. The lymphatics of the mesentery were dilated and contained milk-white chyle. The large intestine was distended by semiformed feces.

The liver was small and congested.

The spleen was enlarged, weighing 350 Gm., and showed intense congestion.

The left kidney had an area of infarction on the posterior surface and a smaller area on the right.

The remaining organs, including the parathyroid, the thyroid and the adrenal glands, the pancreas, the prostate, the testes, the epididymides and the bone marrow, showed nothing of note.

Microscopic Examination.—Sections were taken from the recti abdominis, the gastrocnemius and the intercostal muscles and the diaphragm. The most severely damaged were the recti and the gastrocnemius. Much of their substance was replaced by fat, which was traversed by bands of fibrous tissue. Only small groups or fragments of muscle fibers remained. These showed great variation in size, some being large and edematous, with clumped cytoplasm, and others atrophic (fig. 1). A few fibers were split, but the cross and longitudinal striations were fairly well maintained. Much of the fibrous tissue replacing the muscle was so arranged as to retain the contour of the muscle bundles. The connective tissue fibers were stretched and straight in a few areas, but the majority were curled or wavy. The fibrous tissue was richly vascularized by well formed blood vessels. The blood vessels stood out prominently because they were surrounded by fibrous bands.

Sections of the diaphragm and of the intercostal muscles showed similar but less severe changes.

Numerous sections through the left ventricle of the heart showed normal epicardium. Strands from the subepicardial fat penetrated and isolated the underlying myocardial fibers. The muscle fibers were of varying sizes, many with prominent, richly chromatic nuclei and a large amount of edematous sarcoplasm. Some shrinkage of the large fibers away from the surrounding connective tissue was noted. Many of the large muscle fibers were undergoing degeneration and were surrounded by fibrous tissue. Fibrous tissue replacement of muscle was present in amounts which appeared to equal the original bulk of the muscle. The fibrous tissue was well vascularized by thin-walled blood vessels, and only a few lymphocytes and Anitschkow myocytes were seen in it. Occasional areas of interstitial hemorrhage were observed, but there was no cellular reaction around them. Both the penetration of fat into the myocardium and the replacement of muscle fibers by connective tissue were more noticeable in the region of the epicardium (fig. 2).

Sections through a papillary muscle showed endocardial thickening and large vacuolated myocardial fibers. Large areas of hyalinization were present and appeared older than the majority of lesions described in the left ventricle (fig. 3). There were areas of hemorrhage which were surrounded by many large mononuclear cells with phagocytosed red blood cells. Sections through the right ventricle showed some fibrosis of the epicardium. The fat did not penetrate the myocardium beyond normal limits. A few atrophic muscle fibers were seen, but the majority were hypertrophied.

In the right auricle there was thickening of the endocardium together with some small areas of fibrosis where the muscle fibers had disappeared. The epicardium contained a large hemorrhage, and over this area the fat had been replaced by dense connective tissue.

Staining of tissue removed from the left ventricle for fat revealed a few droplets of varying size in the degenerate muscle fibers. The normal fibers had a faintly orange tint. The fat in the scars stained readily, but the amount of fat seen in the scars was much less than that in the skeletal muscle.

The aorta showed a few atheromatous plaques beneath the intima. The media was thin but regular. The serosa and the vasa vasorum were normal.

The epithelium of the tongue was normal. The muscle bundles were separated by edema. There were variation in size and shape of the muscle fibers and proliferation of the nuclei of the sarcolemma. Striations were persistent even in atrophic or edematous fibers. The amount of fibrous tissue around the muscle was increased and surrounded fragments of degenerate muscle.

The epithelium of the lower part of the esophagus was irregularly desquamated. The underlying lamina propria was sparsely infiltrated by lymphocytes. All the muscle layers were markedly edematous. In the circular layer were a few patches of delicate fibrillar connective tissue (fig. 4). The marked edema of the circular layer brought about some disarrangement of fibers so that many were cut on a different axis from the rest of the muscularis.

The mucosa and the submucosa of the small intestine were normal. Both muscle layers were atrophic, and the fibers were widely separated by edema. Despite this separation, the thickness of the wall was greatly reduced. Because of the edema, many of the fibers were cut on a different axis. The subperitoneal fat was atrophic.

The lungs showed many areas of lipid pneumonia. In one section several small granulomas with foreign body giant cells were arranged around the bronchi. The muscle of the bronchial wall appeared normal as did that of the blood vessels.

Sections of lymph nodes from various parts of the body showed only congestion and edema. One hilar lymph node contained many large fat-laden mononuclear cells.

As to the thymus, there were large lobules composed of thymocytes, but no Hassell's corpuscles were seen.

The prostate showed an increase in glandular tissue in both the median and the lateral lobes. There was edema of the fibrous and smooth muscle tissue. A few striated muscle fibers attached to the gland revealed the same changes as striated muscle elsewhere.

The tubules of the epididymis were separated by edematous connective tissue. Mature spermatozoa were seen in the lumens. The smooth muscle about the tubules was edematous.
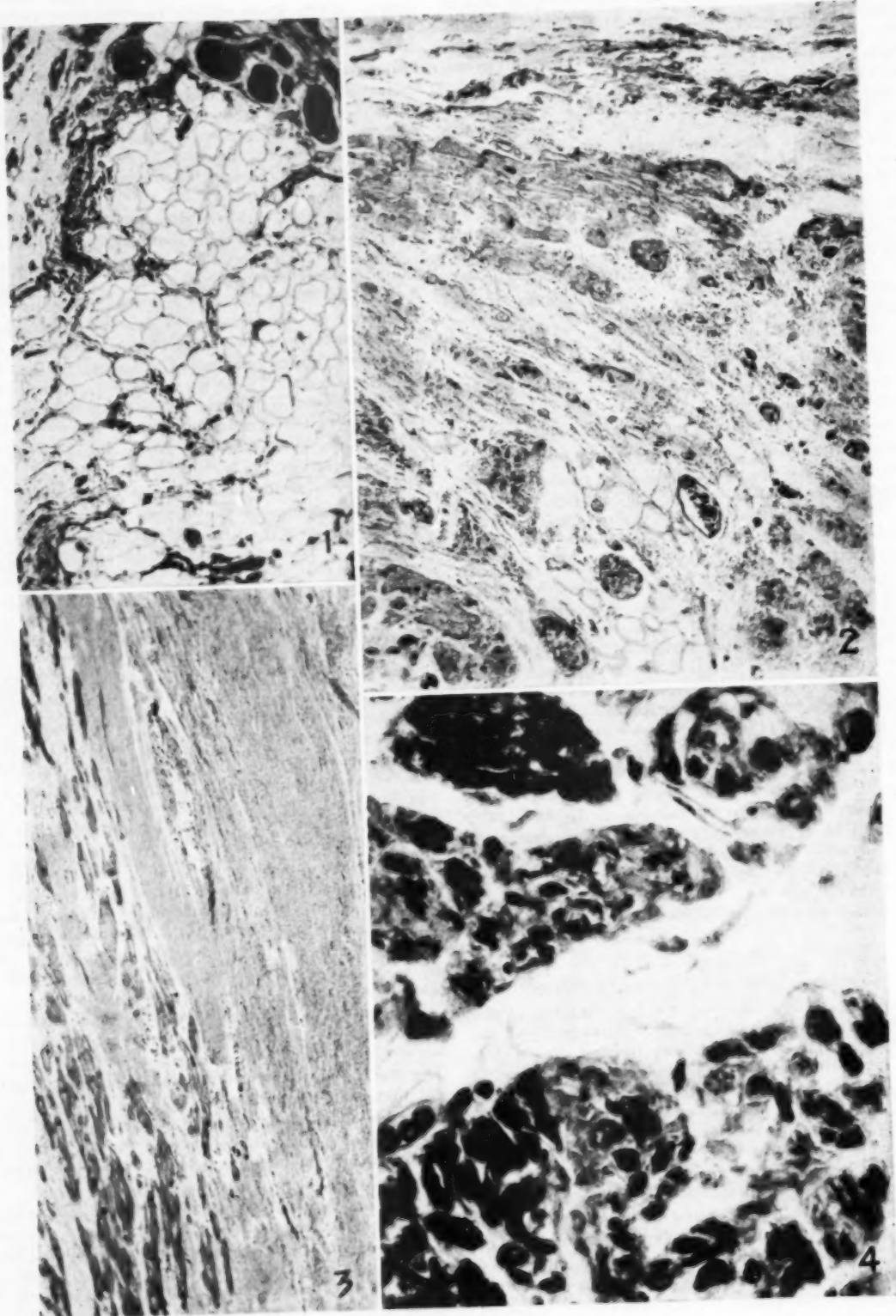
(See legends on opposite page)

The testes appeared normal, but there were few Leydig cells. Those that were seen were devoid of pigment. Spermatogenesis was evident.

The kidneys showed several well defined areas of necrosis surrounded by hemorrhagic zones. The tissues of the adjacent pelvis were thickened and contained an extravasation of red blood cells. The smooth muscle showed no change beyond severe edema.

Sections of adrenal gland, thyroid gland, pancreas, spleen, liver and parathyroid gland showed nothing of note in the present connection.

The anatomic diagnosis included progressive muscular dystrophy, cardiac hypertrophy and dilatation, myocardial degeneration due to muscular dystrophy, pneumonia due to aspiration of lipid with foreign body granulomas, pulmonary congestion and edema, persistent thymus, hypoplasia of the aorta, infarcts of the kidneys, hemorrhagic pyelitis on the left side, congestion of the liver, the spleen and the kidneys and mild adenomatoid hyperplasia of the prostate.

CASE 2.—M. S., a 14 year old white boy, was admitted March 23, 1943 and died July 17, 1944. Weakness of the muscles began at the age of 4. No familial history was obtainable. When the patient was 6 years old, he attended school for one year but could not keep up with the other children and was hospitalized. He remained in various institutions from 1936 until his death. On admission he was well nourished. The lower extremities were held in the typical froglike position. There were bilateral pes equinus, wasting of all the musculature and contracture of the tendons in the region of the elbows, the wrists, the knees and the ankles, as well as wasting of the pectoral and trunk muscles. The shoulder and hip joints were flail-like. The reflexes were absent. Sensation was unimpaired. Only the fingers and the toes could be moved. The patient had to be turned, and if raised to a sitting position, he had to be supported. There was no disturbance in swallowing. The tongue was normal in size and motility. The chest showed marked scoliosis with the convexity to the right. The heart was not enlarged. The rhythm was regular at 80 per minute, and a harsh systolic murmur was heard to the left of the sternum in the second interspace. About two weeks after admission pain developed in the right lower quadrant of the abdomen. The temperature rose to 102 F., the pulse rate to 148 and the respirations to 33. An acutely inflamed appendix was removed with the patient under local anesthesia. The patient made an uneventful recovery. On the day of death he had diarrhea following nine days of constipation; the pulse rate was 140 and the temperature 100 F. One and a half hours later the patient was cyanotic, and white foam poured from his mouth. The ventricular rate was 180 when he died.

Laboratory Data. — A roentgenogram of the chest showed scoliosis, but the heart was not clearly visualized. A single electrocardiogram showed myocardial damage. The Wassermann test was negative. The urine was normal.

The hemoglobin content was 99 per cent; the red blood cell count, 5,260,000; the white cell count, 7,000. The sedimentation rate was 6. The blood sugar during fasting was 100 mg. per hundred cubic centimeters. The urea nitrogen was 11 mg. per hundred cubic centimeters.

Treatment was symptomatic.

Necropsy (eighteen hours after death).—Abnormal mobility of shoulder, hip and vertebral column was noted. The tendons of the arms were contracted. The legs were drawn up in a froglike position, owing to contracture of the tendons. The feet were in the position of pes equinus. Generalized wasting of the skeletal musculature was apparent. The distribution of hair was that of a normal adolescent male.

The pectoral muscles were almost entirely replaced by fat. The intercostal muscles were pale and thin but less affected than the pectoral. The chest was asymmetric because of marked scoliosis with convexity to the right. The trachea was deviated to the right, and the heart was drawn nearer the midline than usual because of the bony deformity. There was 100 cc. of clear amber fluid in the left and 200 cc. in the right pleural spaces.

The lungs were small but heavy in proportion to their size. Large amounts of frothy fluid exuded from the cut surfaces.

The heart weighed 160 Gm. and was small. The epicardial fat was abundant and seemed to invade the right ventricle so that in places no myocardium could be discerned between the epicardium and the endocardium. The endocardium of both ventricles was thicker than normal. The right ventricle measured 0.4 cm. in thickness, in contrast to a thickness of 1 cm. in the left ventricle. There were no lesions of the valves. The myocardium of the left ventricle showed several gray-white streaks. The left main coronary artery and the ascending aorta had a few small atheromatous plaques beneath the intima.

The abdominal panniculus was about 0.3 cm. thick. The musculature was so atrophic that it was difficult to see between the sheaths of the rectus abdominis. There was 50 cc. of fluid in the abdominal cavity. The diaphragm was at the sixth right and the fifth left interspace. The muscle was thin.

The tongue was normal in size and on section showed the usual amount of fat, fibrous and muscle tissue. The esophagus appeared to be normal except for dilatation of the vessels at the cardiac end.

The stomach was extremely dilated, containing about 3 quarts (2,840 cc.) of recently ingested food. The rugae were flattened. A nodule covered with mucosa projected about 0.5 cm. above the surface in the prepyloric region. The small intestine was of normal caliber,

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EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Gastrocnemius muscle (case 1) showing the variation in size of the remaining muscle fibers. Note the widespread replacement with fat and connective tissue. The only remaining muscle fibers are seen at the top. Phosphotungstic acid and hematoxylin; × 184.

Fig. 2.—Section of the left ventricle (case 1) showing severe fibrosis in the outer layers of the myocardium. Note the fat deposits in the fibrous tissue in the lower part of the section. Hematoxylin and eosin; × 194.

Fig. 3.—Papillary muscle (case 1) with dense hyalinized scar tissue and a normal small artery. Hematoxylin and eosin; × 155.

Fig. 4.—High power view of the smooth muscle of the lower part of the esophagus (case 1) showing replacement by fibrous tissue. Note the variation in size and the disintegration of some of the muscle fibers. Trichrome stain; × 824.
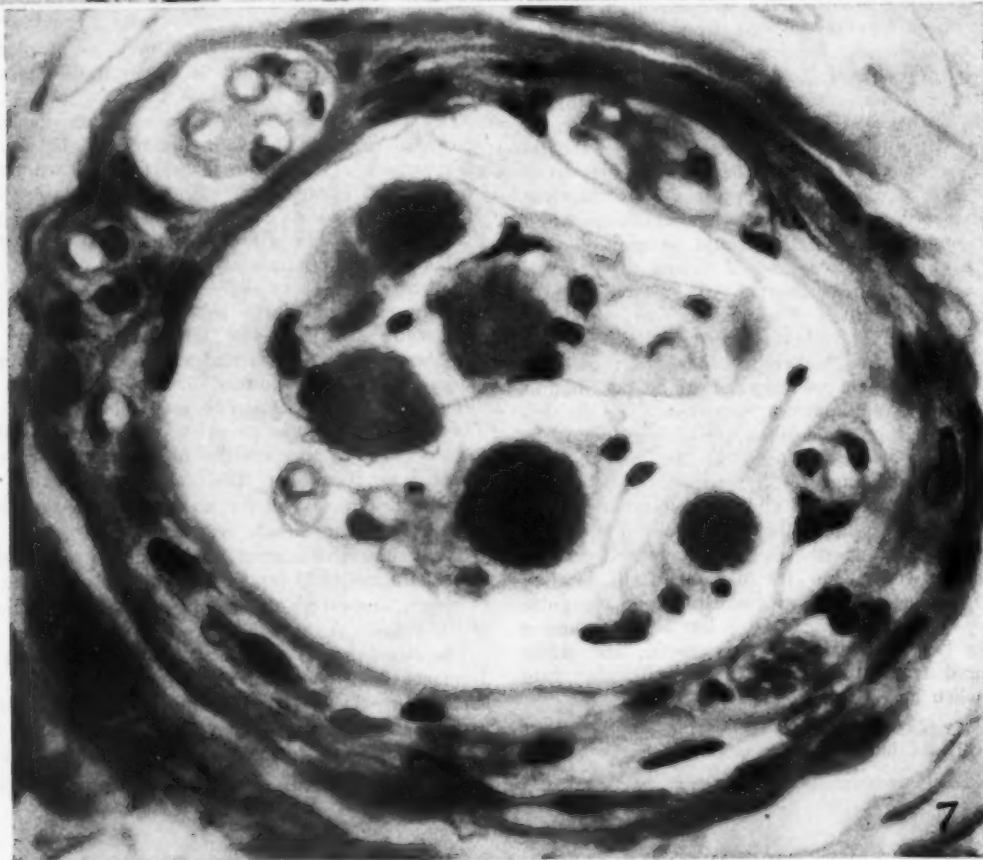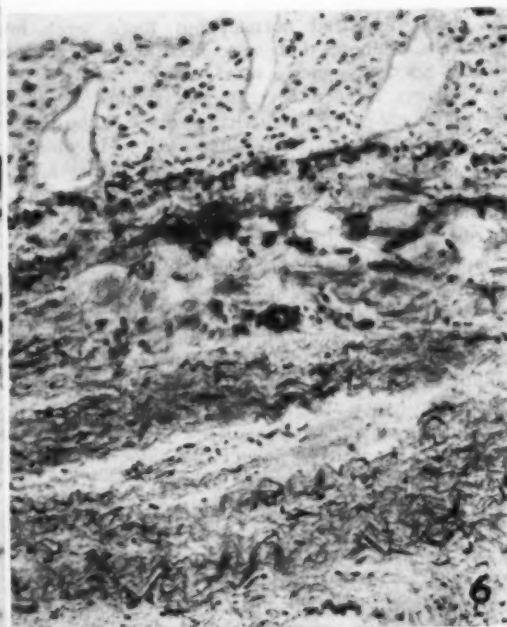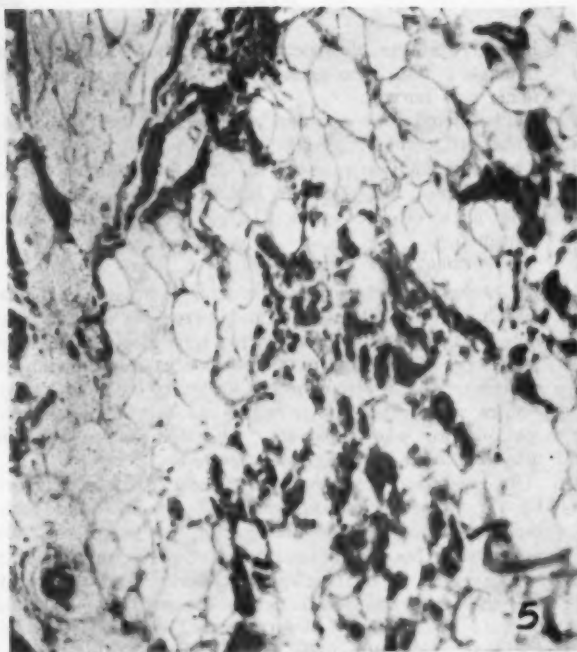
Fig. 5.—Right ventricle (case 2) showing isolation of groups of myocardial fibers by fat. The fibers are atrophic and fragmented. Hematoxylin and eosin; × 160.

Fig. 6.—Wall of rectum (case 2) showing a small amount of cellular infiltration extending from the mucosa into the submucosa. Note the stretched circular muscle layer in which there are large spaces. The longitudinal layer is atrophic and the fibers are spread apart. Hematoxylin and eosin; × 160.

Fig. 7.—Large channel-like structure in psoas muscle (case 3) showing disintegrated muscle fibers in the lumen and capillaries in the wall. Such structures probably represent perimysium persisting after the disintegration of muscle cells. Hematoxylin and eosin; × 880.

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and the contents were bile stained. The colon was greatly dilated and was filled with liquid and formed fecal material. At the rectosigmoid junction a mass of feces covered a hemorrhagic mucosa. From this region to the anus, stony-hard fecal material distended the colon. The musculature did not appear altered.

The liver contained large anomalous blood channels, which were cystlike on palpation. The extrahepatic portions of the portal and hepatic veins were not abnormal.

The remaining organs, including the brain, the pancreas, the adrenal, pituitary, thyroid and prostate glands, the testes, the epididymides, the spleen and the kidneys, were normal.

Microscopic Examination.—Sections of the diaphragm and of the psoas, rectus abdominis, pectoral and intercostal muscles showed lesions which were practically identical with those described in the striated muscle of the patient in case 1. Microscopically, the most extensively damaged muscle was the pectoral.

The epicardium varied from normal to complete replacement by fibrous tissue. In some instances the underlying myocardium was similarly replaced; in other instances it was unchanged. There was no fat in the areas of scarring, nor any cellular reaction. In places collections of fat were seen some distance beneath the myocardial fibers and the epicardial surfaces. In the fat were many blood vessels surrounded by bands of connective tissue. The myocardial fibers were small, but there were occasional areas where they varied in size and were fragmented and the nuclei distorted. The nuclei of the connective tissue surrounding the fibers were more numerous than in other areas. The endocardium was normal or slightly thickened.

Sections of the right side of the heart showed extensive fat infiltration, the fat penetrating from the epicardium to isolate small groups of myocardial fibers. These muscle fibers were fragmented and atrophic, and many had lost their transverse striations (fig. 5). Section at the right side of the apex showed almost no muscle tissue. With fat stains a few minute intramuscular globules were seen. There was no increase in perinuclear lipochrome. The coronary arteries were normal but were surrounded by thick bands of fibrous tissue. Fat was interspersed in the muscle tissue through the entire thickness of the ventricle, and there were collections of fat beneath the endocardial lining.

The aorta had small areas of atheromatous thickening beneath the intimal lining. The other coats of the aorta and the vasa vasorum were normal.

The epithelium of the tongue was normal. The lamina propria was not increased in thickness. The striated muscle showed many swollen and fragmented fibers, loss of striation and proliferation of the nuclei of the sarcolemma. The amount of fibrous tissue replacement was small. The blood vessels were not unusual.

The mucosa of the upper part of the esophagus was intact. The inner longitudinal muscle coat was well defined. The circular muscle layer was composed of both striated and smooth muscle. The striated fibers displayed great variation in size, and there was a large amount of connective tissue between them. Many of the fibers were fragmented and showed an increase of nuclei at the periphery. Such changes were even more pronounced in the outer longitudinal layer. There was great variation in size. Small bundles of smooth, wavy muscle were also present. The muscle fibers of the longitudinal layer were fragmented, and many were split. Areas of fibrosis were continuous with the degenerate fibers. There was some lymphocytic infiltration in the longitudinal muscle layer in addition to proliferation of nuclei of the sarcolemma.

The lower part of the esophagus showed smooth muscle which appeared well preserved except for some edema which made the muscle bundles that were cut in a longitudinal direction appear wavy. Occasional small scars were seen in the outer longitudinal layer.

The mucosa in one section of the stomach had a small pancreatic rest lying beneath it. Sections from the prepyloric region showed fragmentation of the muscularis mucosa. The muscularis was edematous. There seemed to be a slight increase of fibrous tissue, which penetrated from the serosal surface. The outer muscle layers were thin and the muscle fibers disarranged. Despite the gross dilatation of the stomach, the wall appeared of usual thickness.

The lymphoid tissue in the mucosa of the small intestine was abundant. Both muscle layers were greatly atrophied. There was no fibrosis, but the longitudinal layer was wavy.

The rectal mucosa was autolyzed and infiltrated throughout by red blood cells. A few polymorphonuclear cells were seen in surrounding areas and extended into the edematous submucosa. The muscularis mucosa was fragmented. The longitudinal muscle layer was extremely thin. The nuclei appeared long, wavy and sometimes comma shaped. The muscle fibers were disintegrated and stretched. The circular layer was also markedly atrophic and the muscle fibers widely separated. There were large spaces due to loss of muscle tissue. In these spaces were small spherical bodies of amorphous pink material which appeared to be products of disintegration of the muscle fibers (fig. 6).

No lesions of the blood vessels were seen in the gastrointestinal tract, and the plexuses of Auerbach were normal.

The lungs were strikingly congested. The alveolar septums were thin and delicate, and the air vesicles contained fluid.

Lymph nodes from various parts of the body appeared to be normal.

The liver was markedly congested in the areas of the central veins. Several huge blood channels lined with endothelium were seen, the walls of which had the structure of veins. Each small portal vein was slightly dilated.

The brain, the pancreas and the thyroid, pituitary, adrenal and prostate glands appeared to be normal.

The epididymis had only a little pigment in the lining epithelium. The smooth muscle of the walls of the tubules appeared to be normal.

In the testes the tubules appeared to be normal. There were abundant Sertoli cells and widespread active spermatogenesis. The interstitial tissue was scant. The Leydig cells were few, and none contained pigment.

The kidneys appeared to be normal except for the pelvis of one, which showed some edema of the walls.

The anatomic diagnosis included progressive muscular dystrophy with involvement of the myocardium and the gastrointestinal tract, marked scoliosis with asymmetry of the chest, dilatation of the right ventricle of the heart, acute pulmonary edema, pleural effusion and ascites, hypoplastic aorta, dilatation of the stomach and of the colon, fecal impaction, anomalous veins of the liver, a pancreatic rest in the stomach, bilateral pes equinus and tendon contractures of the extremities.

CASE 3.—A. S., a 16 year old Negro boy, was admitted Jan. 6, 1942 and died Dec. 13, 1942. Progressive muscular dystrophy was diagnosed at 6 months of age. He had been hospitalized for nine years prior to his admission and had been confined to a chair for eight of these years. An older brother died of progressive muscular dystrophy at the age of 14, having had the disease since infancy.

Two older brothers were apparently free of any form of muscle disease. On admission the patient was unable to lift his head. There were flexion contraction of the elbows and the knees and weakness of the shoulder muscles, also bilateral pes cavus. The thoracic muscles, the sternocleidomastoid muscles and all the muscles of the upper extremities showed marked weakness. Reflexes were absent. There were flattening of the chest in the anteroposterior diameter and curvature of the dorsal part of the spinal column to the left. The heart was not enlarged to percussion. The blood pressure was 100 systolic and 70 diastolic. In March, about two months after admission, the patient had a sudden rise in temperature to 103 F. The ventricular rate was 160. There was dulness on the left side of the chest, as well as diminished breath sounds over the bases of the lungs. The white cell count rose to 21,000, 92 per cent of which were polymorphonuclear leukocytes. After the acute episode, the patient continued to cough for several weeks and produced white frothy sputum. After two months there was another acute episode, and though no consolidation was seen in roentgenograms the diagnosis of pneumonia was made. During the next few months there were at least five acute attacks, during all of which the pulse rate ranged from 120 to 170. Pressure on the carotid sinus did not reduce the pulse rate, but in September small doses of digitalis established a pulse rate of 73. Late in October a particularly severe attack of pneumonitis necessitated confinement in a respirator, and on December 13 death occurred. During the last month digitalis was without apparent effect on the pulse rate.

Laboratory Data.—The basal metabolism rate was —15 and —10 per cent of the normal standard on November 19 and 23. A tuberculin test was negative. Numerous electrocardiograms showed sinal tachycardia. The electrical axis changed from no deviation on admission to right deviation in May, and this persisted until death. Roentgenograms of the skull were normal; those of the knees and the elbows showed thinning of the diaphyses with normal development of epiphyses and epiphysial lines. Osteoporosis of the distal ends of the carpal and tarsal bones and the bones of the pelvis was seen. There were curvature of the dorsal part of the spinal column to the left and elevation of the right side of the diaphragm. A roentgenogram of the chest November 18 showed consolidation. Esophagrams taken at the same time disclosed no displacement and no indentation from the heart. There were no varices or obstruction. The dextrose tolerance test gave a normal result. On December 7 the Cutler-Power-Wilder test for adrenal function was inconclusive.

Treatment consisted in maintaining the patient on a high caloric, high vitamin diet and wheat germ oil, and administering bile salts, thiamine, nicotinic acid and aminoacetic acid.

Necropsy (twenty-four hours after death).—General wasting of the muscles was apparent. The legs were in a froglike position, and there were bilateral deformities of the feet of the pes cavus type.

Scoliosis of the spinal column with convexity to the left and corresponding deformity of the right side of the chest were present. The pectoral muscles to a great extent were replaced by fat. There was no fluid in the pleural cavity, but the pleura covering the lower lobes of the lungs showed fibrous tags. The lungs were heavy, and through the pleura yellow patches were visible. The entire lower lobe of the right lung and a large part of the lower lobe of the left lung were consolidated. The surfaces of the consolidated areas were yellow-red. In the remaining lobes consolidation was localized around the bronchi. The cut surfaces were dotted with yellow spongy areas. The bronchi and the trachea contained large quantities of mucopurulent material.

The heart weighed 140 Gm. In the epicardium over the anterior surface of the right ventricle, which was slightly dilated, there was an opaque area about 2 by 5 cm. The endocardium was thickened over the trabeculae carneae. The myocardium was not hypertrophied, but streaks of fibrous tissue were seen in the left ventricle and in the interventricular septum. The valves were normal. The coronary arteries were free of sclerosis.

The aorta showed several small atheromatous plaques. The musculature of the abdominal wall was thin. The diaphragm was so thin as to be translucent.

The tongue was of normal size. The muscle appeared well developed, and the amount of fat was not excessive. The pharynx and the upper part of the esophagus were normal. The muscularis of the lower part of the esophagus was thicker than usual. The stomach was small, and the rugae were well preserved. The walls were of usual thickness. The duodenum was slightly dilated. The walls of the remainder of the small intestine, although not dilated, were thinner than normal. The rectum was dilated, but the walls were of normal thickness and the muscle layers were well differentiated.

All other organs examined, including the brain, the spleen, the kidneys, the pancreas, the adrenal glands, the pituitary gland, the prostate, the testes and the bladder, were normal.

Microscopic Examination.—Of the striated muscles, the psoas presented numerous areas of degeneration, with infiltrating lymphocytes and marked proliferation of the nuclei of the sarcolemma. Many muscle fibers had disappeared, leaving vacuoles. In these areas were well preserved small blood vessels. There were peculiar channel-like fibrous structures, which appeared to surround fragments of degenerate muscle. These channel-like structures were lined by a single layer of flat cells. In their walls were a few capillaries containing red blood cells (fig. 7). With trichrome stains no smooth muscle could be found in the walls of these structures. The blood vessels, the fibrous tissue and the muscle cells showed the same changes that have been described in the other patients. Sections of the diaphragm and of the pectoral and intercostal muscles revealed varying degrees of damage. The pectoral muscle was the most extensively involved.

As to the heart, numerous sections of the left ventricle and of the interventricular septum showed areas where the epicardial fat was replaced by scar tissue. The scar tissue penetrated and replaced the myocardium so that more fibrosis was evident in the outer layers of the myocardium than toward the endocardial surface. The scar tissue was diffusely though sparsely infiltrated with wandering cells and lymphocytes. The myocardial fibers were degenerate, and those adjacent to the areas of scarring appeared to be hypertrophied and somewhat edematous. Their striations were fairly well maintained. In the areas of fibrosis were occasional clumps of light brown refractile pigment indistinguishable from lipochrome. There was little fat in the scar tissue, although aggregates of three to four fat cells were sometimes present (fig. 8). With sudan III stains no increase of fat in the muscle fibers was seen. The endocardium in a few small areas was thickened by fibrous tissue.

In the right ventricle the abundant epicardial fat penetrated the myocardium to isolate groups of muscle fibers and single fibers. Many of these fibers were fragmented and atrophic. Others had a wavy outline with many undulations. There were a few small areas of fibrosis,

but these were insignificant as compared with those seen in the left ventricle. The epicardium was thickened in a few areas, but the endocardium was normal.

Throughout all sections the blood vessels were normal even when they were in the midst of scar tissue.

The aorta showed atheromatous deposits in the intima which were so thick as to compress the media.

The tongue had normal epithelium. The fibrous tissue of the lamina propria did not exceed the usual amount. The majority of the muscle fibers appeared to be normal, but there were areas where the fibers were undergoing degeneration and the nuclei of the sarcolemma had proliferated. In one place the muscle fibers seemed to have disappeared. There was almost no cellular infiltration

changes similar to those described elsewhere (fig. 9). The changes were more severe than those seen in the tongue.

The lamina propria of the lower part of the esophagus was densely infiltrated with a few polymorphonuclear leukocytes, lymphocytes and wandering cells. There was generalized edema. Both the circular and the longitudinal muscle layer showed many vacuolated areas. The cells which remained were edematous and varied in size. There was no cellular reaction or replacement fibrosis in the muscle layers. The plexuses of Auerbach and Meissner were edematous but otherwise normal.

The mucosa of the stomach was well preserved. The muscularis was reduced to a few wavy, fragmented and
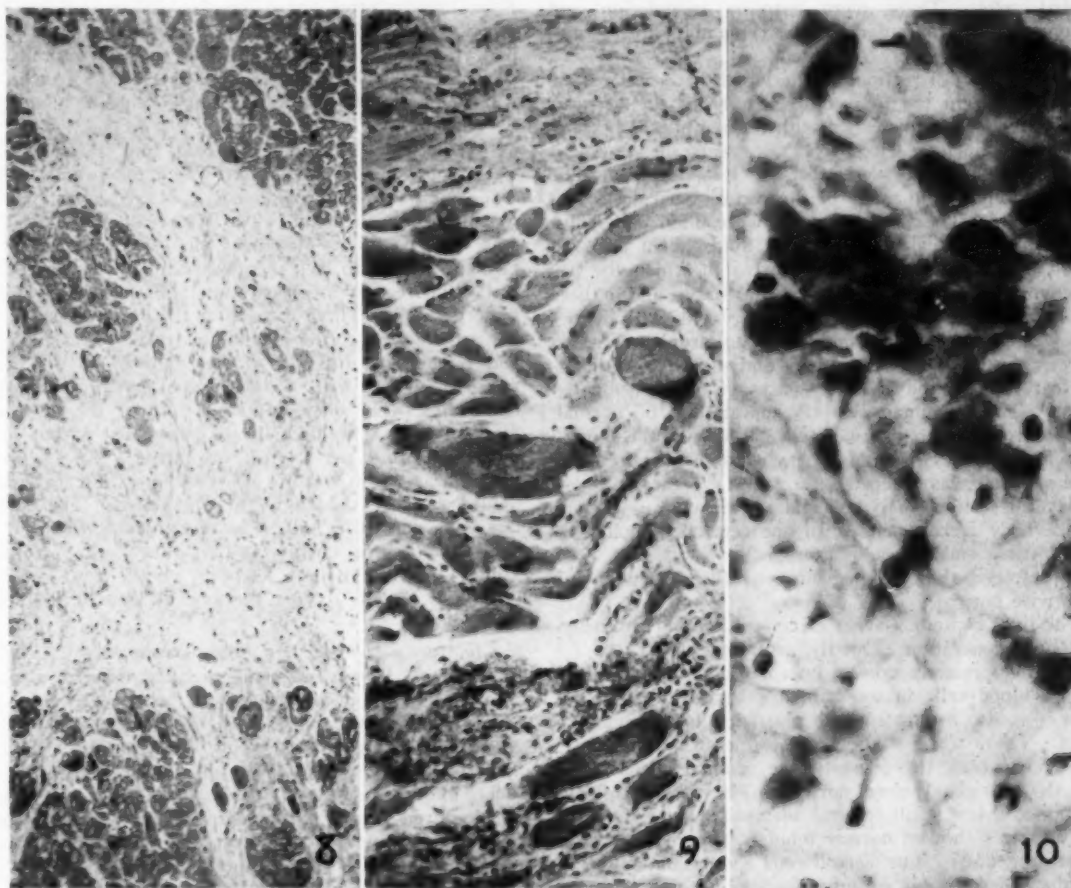

Fig. 8.—An area of myocardial scarring in the left ventricle (case 3). Hematoxylin and eosin; × 160.
Fig. 9.—Upper part of the esophagus (case 3). Note the variation in size of the muscle fibers, the replacement by fibrous tissue and the mild cellular infiltration in the latter. Hematoxylin and eosin; × 160.
Fig. 10.—High magnification of the smooth muscle in the wall of the stomach (case 3) showing vacuolated areas, variation in the size of the fibers and disintegration of the fibers. Hematoxylin and eosin; × 800.

about these areas. The small blood vessels were prominent, but their walls were normal.

The pharynx presented areas of muscle degeneration similar to those seen in the tongue.

The upper part of the esophagus showed generalized edema and lymphocytic infiltration of the lamina propria. The mucosa was intact. The inner longitudinal muscle layer was fragmented and varied in thickness. The circular smooth muscle was atrophic. Many fibers appeared to have dropped out; others were vacuolated. The striated muscle of both layers had undergone

poorly stained fibers. The submucosa was congested. All the muscle layers were extremely atrophic. Many fibers were missing. There was variation in the size of the fibers, but all had indefinite outlines. Many of the nuclei had disappeared; other nuclei were fragmented (fig. 10).

Less damage was found in the muscularis of the duodenum than in that of the stomach. However, a slightly increased amount of fibrous tissue was demonstrable with trichrome stain in the longitudinal layer in sections cut in both the longitudinal and the transverse direction.

Some of the muscle fibers were cut in different planes. The plexuses appeared unduly prominent, probably owing to edema and to atrophy of the muscularis.

Sections of the large intestine and the rectum showed the most marked changes in the latter. In one section there was early infarction of the mucosa, and the veins were occluded by thrombotic material; the walls of the veins were necrotic and infiltrated with polymorphonuclear leukocytes. Colonies of bacteria were seen in the mucosa and the muscularis. The latter was edematous and infiltrated with polymorphonuclear leukocytes. In other sections there was no evidence of an inflammatory reaction. Many mucosal glands had been replaced by fibrous tissue, and in it were clumps of nonrefractile brown pigment. Some of this pigment had been phagocytosed by mononuclear cells; some was present in epithelial cells. There were extreme vacuolation and atrophy of the muscularis. The remaining fibrils were edematous and widely separated.

Throughout all sections of the lungs there was patchy consolidation in the immediate vicinity of the bronchi. The bronchi were distended with mucus, in which many polymorphonuclear leukocytes were enmeshed. About the larger bronchi were foci of lymphocytes. The alveolar septums in many of the consolidated areas had disappeared. The alveolar spaces were filled with polymorphonuclear leukocytes, edema fluid, fibrin and red blood cells. There were large areas in which the parenchyma had been replaced by fibrous tissue, and in these areas were many large mononuclear cells with foamy cytoplasm. Fat stains showed large droplets free in fibrous tissue, in the foam cells and also in the mononuclear cells in the alveolar spaces of the consolidated areas. The pulmonary vessels appeared to be normal. The walls of the bronchi were edematous, and the smooth muscle fibers were spread apart, but no other alteration in the muscle was observed.

Several sections of lymph nodes from various regions of the body were normal. In one cervical lymph node there was a small area of necrosis.

The spleen showed congestion of the pulp but was otherwise normal.

The liver contained areas of congestion and parenchymatous degeneration about the central veins.

The kidneys were normal except for an extravasation of red blood cells in the lamina propria of the pelvis. This was also infiltrated with plasma cells and lymphocytes. There was no lesion in the smooth muscle.

The pancreas, the prostate, the seminal vesicles and the thyroid gland were normal. The adrenal glands contained a few small cortical adenomas.

The testes showed normal tubules. There was active spermatogenesis. The Sertoli cells were abundant. The Leydig cells were not prominent and did not contain pigment. The smooth muscle was scant but appeared normal. Fragments of the cremasteric muscle showed atrophy of a few fibers and some proliferation of the nuclei of the sarcolemma.

The anatomic diagnosis included: progressive muscular dystrophy involving the myocardium, the skeletal muscles and the gastrointestinal tract; kyphoscoliosis and asymmetry of the chest; bilateral pes cavus; pneumonia due to aspiration of lipid in the middle and lower lobes of the right lung and in the upper and lower lobes of the left lung; infarction of the mucosa of the large intestine; arteriosclerosis of the aorta, chronic passive congestion of the liver and melanosis coli.

CASE 4.—F. S., an 18 year old white youth, was admitted March 19, 1940 and died Aug. 14, 1940. The onset of muscular disease occurred at the age of 5 years, but the patient attended school until he was 10 years old.

At that time the disease had progressed so that hospitalization was necessary. His appetite had always been poor and the bowels constipated. He was the younger of two sons, who were the only children in the family. There was no history of the disease in either parent's family. On admission there was marked wasting of all muscles except the gastrocnemii. The hips were rotated outward. There was permanent flexion of the knees, the elbows and the wrists due to contracture of the tendons. There was bilateral equinovarus. The lower jaw was prominent. Scoliosis with the convexity to the left and lumbar lordosis were marked. The heart seemed to be enlarged but was difficult to percuss because of the deformity of the chest. The blood pressure was 110 systolic and 70 diastolic. The pulse rate was 90, and there was sinal arrhythmia. The lungs were clear. Four months after admission the patient had the first attack of diarrhea, which was controlled with camphorated tincture of opium. Two weeks later diarrhea recurred. No blood or excess of mucus was seen in the watery stools. The patient had no fever, abdominal pain or tenesmus. One month after the second episode he experienced severe abdominal pain, and impacted feces were removed manually, after which the pain subsided. The following day abdominal pain recurred and the patient began to vomit. The temperature at this time was 104 F. The abdomen was distended but soft, probably owing to lack of musculature in the abdominal wall. There was increased peristalsis with many loose, watery stools. Three days after this, on August 7, the patient vomited coffee ground material. The abdomen was tender and distended. The white blood cell count rose to 20,450 with 85 per cent polymorphonuclear leukocytes. The urine contained acetone (4 plus) and diacetic acid. The diagnosis of perforated gastric ulcer with spreading peritonitis was made. At operation multiple areas of necrosis were seen in the greatly distended stomach, which were invaginated. The patient did well until the fourth postoperative day, when he suddenly became comatose. At this time there was dulness over the left of the chest, with marked cyanosis. Despite oxygen therapy, the patient died three hours later.

Laboratory Data.—No electrocardiographic studies were done. Roentgenograms revealed uniform atrophy of all bones and decalcification of the lower ends of the femurs and of the hands and the wrists. The lungs showed no abnormalities. The heart was not enlarged. The roentgenograms of the skull were normal.

The blood creatinine ranged from 2.4 mg. to 3.9 mg. per hundred cubic centimeters. The blood sugar was 69 mg. per hundred cubic centimeters. The red blood cell count was 3,896,000, the hemoglobin content, 96 per cent. The Wassermann test was negative.

Treatment included administration of wheat germ oil and vitamins by mouth and by infusion, including vitamins C, B₁ and K and nicotinic acid.

Necropsy (twenty hours after death).—There was moderate prognathism. Bilateral equinovarus and outward rotation of the hips were noted. The musculature of all the extremities and of the trunk was wasted.

There was asymmetry of the chest due to scoliosis with convexity to the left, which diminished the space on that side. Each pleural space contained 500 c.. of thin straw-colored fluid. The lower lobes of both lungs were compressed and firmer than usual.

The heart weighed 140 Gm. No hypertrophy, dilatation or myocardial fibrosis was noted. The coronary arteries were free of sclerosis.

The esophagus was normal. The stomach was discolored. Sutures were present over the anterior sur-

face and the greater curvature. The wall was invaginated in these areas and on section was necrotic. In several places hemorrhagic areas measuring 1 to 3 cm. in diameter, with beginning ulceration, were demarcated from the mucosal surface. The remainder of the gastrointestinal tract was described as normal.

The liver was small and congested.

The remaining organs examined, including the brain, the spleen, the pancreas, the adrenal glands, the pituitary gland, the testes, the prostate and the bladder, were normal.

Microscopic Examination.—Sections of the intercostal and latissimus dorsi muscles showed the same changes as those described in the previous cases of this series. The same sort of channels containing fragments of muscle were seen as in case 3. There was widespread replacement of muscle by fat and connective tissue.

Numerous sections of the left ventricle of the heart showed connective tissue replacing the epicardial fat and scar tissue penetrating into the myocardium. In many small areas active myocardial degeneration was evident. This was characterized by fragmentation, loss of striation, clumping of cytoplasm and disintegration of muscle fibers, presence of edema both of muscle and interstitial tissue, loss of nuclei, proliferation of the so-called sarcolemma or covering of individual muscle fibers and presence of a few lymphocytes and wandering cells. The absence of large numbers of thin-walled blood vessels such as were seen in the areas of scar tissue was noteworthy. The transition of these areas into the more common densely scarred areas could be traced. The older areas were well vascularized by thin-walled congested vessels and there were small aggregates of fat cells in the scar tissue. Several of the trabeculae carneae were partially replaced by fibrous tissue. With trichrome stains, only small amounts of fibrous tissue could be seen. The appearance of fibrosis was due to empty shells of muscle fibers and numerous interfascicular capillary walls. With stains for connective tissue the predominantly epicardial position of the fibrosis was strikingly demonstrated. Much of it seemed to be derived from perivascular bands of connective tissue in the epicardium.

The right ventricle showed much less damage than the left. There were a few small, atrophic fibers, with some tendency toward "ribboning." The blood vessels were surrounded by bands of connective tissue. The epicardial fat was abundant but normal.

The esophagus was so edematous that the walls were at least twice as thick as normal. The mucosa was intact. The lamina propria was pulled apart so that only fragments of fibrous tissue remained. The inner longitudinal muscle was also fragmented. In both the outer longitudinal and the circular muscle layers there was a small amount of scarring. In many places where the muscle cells had dropped out, no replacement fibrosis was present. Great variation in the size of the muscle fibers was noted.

The lesions of the stomach have been described in detail elsewhere.[1] All the changes observed in the smooth muscle in the preceding cases were present with the additional features of inflammation and perforation. In sections removed far from the area of perforation the mucosa was intact. The muscle layers were edematous and infiltrated with lymphocytes and wandering cells. In both the muscularis and the serosa there were several small areas of scarring, the seats of previous damage. The serosa was infiltrated with polymorphonuclear leukocytes, lymphocytes and wandering cells.

The duodenum showed marked edema of the submucosa. Both layers of the muscularis were atrophic.

In the latter were many large spaces which contained fragments of disintegrating muscle and many nuclei. A few wandering cells and lymphocytes were seen. The serosa was not unusual.

In the lungs, many of the air spaces were collapsed. Others were shrunken, and most of them were filled with edema fluid and red blood cells. The bronchi contained edema fluid, but the walls were normal. The blood vessels were normal.

The liver showed chronic passive congestion. In the liver cells, especially those about the portal radicles, there were large vacuolated areas.

The remaining organs examined, including the pituitary and adrenal glands, the pancreas, the kidneys and the brain, were normal.

The anatomic diagnosis included progressive muscular dystrophy with myocardial degeneration, acute phlegmonous gastritis with perforation, acute generalized peritonitis, asymmetry of the chest due to scoliosis, bilateral pleural effusion, partial atelectasis of the lower lobes of the right and left lungs, pulmonary edema, chronic passive congestion with fatty metamorphosis of the liver, bilateral equinovarus and a recent laparotomy wound.

COMMENT

The changes in the voluntary muscles were those of progressive muscular dystrophy as described by many observers. They consisted of great variation in the size and the shape of the fibers, vacuolation and clumping of the sarcoplasm, waviness of the fibers and proliferation of nuclei of the sarcolemma. Despite these structural alterations, the striations were remarkably well preserved. The muscle was eventually replaced by fibrous tissue and fat, which, however, did not appear to penetrate from the surrounding fascia or the perimysium. One of the striking features noted in the skeletal muscles but not prominent elsewhere was the presence of channel-like structures the lumens of which contained fragments of degenerate muscle. These structures probably represent the remaining perimysium. Cellular reaction was slight or absent throughout.

All 4 patients had myocardial lesions. Only 1 patient (case 1) revealed clinical signs of heart failure. All of them had tachycardia. The electrocardiograms for 2 patients showed deviation of the electric axis to the right. In connection with the last-mentioned finding deformities of the chest must be taken into account.

Anatomic and histologic examination revealed the most severe myocardial lesions in a patient who had been in cardiac decompensation. This was the only patient in whom hypertrophy of the heart was noted. Next in severity was tachycardia in the patient (case 3) whose cardiac disturbance was controlled for a time with digitalis. The third was the patient (case 4) in whom the signs of cardiac arrhythmia were overshadowed by gastrointestinal symptoms. Least damaged

was the myocardium of the patient (case 2) whose persistent tachycardia and harsh systolic murmur at the apex suggested a cardiac lesion. In 3 of the 4 patients the myocardium of the left ventricle was more severely damaged than that of the right ventricle.

A composite picture of the gross myocardial lesions showed increase in and opacity of the epicardial fat, streaks of fibrosis throughout the myocardium, particularly in the left ventricle, and slight focal thickening of the endocardium. Microscopic examination revealed a peculiar distribution of the scarring and of the areas of muscle degeneration. The most extensive lesions were near the epicardial surface, where the fibrous tissue, which penetrated and replaced the myocardium, appeared to be continuous with the epicardial fat or with the thickened epicardium. The amount of fat in the scar tissue was small. With sudan stains the fat in the myocardial fibers was found to be less than that in many of the control sections. The myocardial fibers showed changes which were comparable to those seen in the skeletal muscles, although there were certain differences. The lesions of the myocardium were less extensive but appeared fresher and their evolution into scars could be traced. Probably the fact that tissues for biopsy in progressive muscular dystrophy are taken frequently from skeletal muscles which not only are severely damaged but which represent the oldest lesions accounts for this difference. The amount of fat within the scar tissue was considerably less than that encountered in the skeletal muscle.

All 4 patients showed abnormalities of the gastrointestinal tract. The striated muscle of the tongue in 3 of the 4 patients showed changes which were milder but similar to those of voluntary muscle fibers elsewhere. The amounts of fat and fibrous tissue in the tongue vary so greatly in normal persons that it is difficult to estimate the average amounts. In the tongues of our patients the only method used to estimate the increase was to note the amount in the vicinity of persisting fragments of disintegrated muscle.

No gross lesions were seen in the esophagus except for an increase in thickness of the wall in 2 cases. The striated muscle in the upper part of the esophagus in 2 cases in which it was examined showed lesions similiar to those in striated muscle elsewhere. The changes in both the tongue and the esophageal muscles were mild compared with those in the skeletal muscle. In the lower end of the esophagus there was marked edema of all layers, but particularly noticeable were the edema and coincidental changes in the smooth muscle layers. The

muscle fibers varied in size and were indistinct in outline. Many had disappeared. The amount of fibrous tissue replacement was small in contrast with the widespread edema. The fibers which remained varied greatly in size and in staining qualities. They were often disarranged and appeared at right angles to the majority of the fibers in the same layer. The cytoplasm was vacuolated, and that in the large fibers appeared to be coagulated. The fibers of muscle cut longitudinally were wavy and extended in long ribbonlike bands; the nuclei were stretched into thin, wavy filaments. There was no cellular infiltration of the muscularis.

In 2 cases the stomach was greatly dilated. Sections of the stomach were available in 3 of the 4 cases. In case 4 sections of the stomach both in and at a distance from the area of perforation showed the described changes in the muscle layer. It seems possible that atony or weakening of the muscle fibers contributed to perforation. The amount of inflammatory reaction so distorted the picture that it was impossible intelligently to estimate the extent of any muscle changes that might have existed previously. In case 2 there was thinning of the outer muscle layer, but edema of the other layers compensated so well that the wall appeared to be of normal thickness.

The changes in the small intestine in case 4 were those of edema, atrophy and disappearance of muscle fibers. In this case, however, the alterations produced by generalized peritonitis must be considered. In the other cases atrophy predominated. In general the changes were less severe than those in the remainder of the intestinal tract, although thinning of the walls was commented on in the gross description of the intestine in case 3.

In the large intestine the rectal lesions were the severest of all. Two of the patients showed extreme atrophy of muscle. In 1 of these (case 2) at autopsy there were fecal impaction and hemorrhage of the rectal mucosa. Incidentally, another patient in this institution with progressive muscular dystrophy had recurrent attacks of abdominal pain, vomiting, diarrhea and fecal impaction for three years before death. Unfortunately, permission for necropsy was not granted. Globus [5] observed fecal impaction in a patient who came to necropsy.

I do not wish to imply that the changes described in the smooth muscle of the gastrointestinal tract are pathognomonic of progressive muscular dystrophy, since severe edema and atrophy may be seen in aged or debilitated patients or in patients with nutritional disturbances. I wish

merely to call attention to the fact that edema and atrophy may account for some of the symptoms which patients with progressive muscular dystrophy exhibit, namely, recurrent attacks of abdominal pain, vomiting, diarrhea and fecal impaction. The spreading apart of the muscle fibers which has been described as due to edema may be a manifestation of atony. Also, it is possible that the variation in size of the muscle fibers may be due, in part, to different degrees of contractility. In any event, these two conditions seemed more pronounced than those in control sections taken from persons with other long-standing debilitating diseases.

The lesions seem to follow the same pattern as those observed in the skeletal and the voluntary muscle and the striated muscle of the myocardium. There were disarrangement, edema, waviness, fragmentation, atrophy, disappearance and finally a small amount of scarring of the muscularis. The absence of cellular reaction was noted in lesions of the striated and of the smooth muscle tissues of the gastrointestinal tract.

The plexuses of Auerbach and Meissner were not unusual except for the fact that they shared in the generalized edema.

The alterations of the smooth muscle in the gastrointestinal tract were much less severe than those seen in scleroderma,[16] and the fibrous tissue replacement was less prominent. Since Kuré[3] stressed the role of the autonomic nervous system in both diseases, the comparison is warranted.

The aorta appeared to be hypoplastic in 2 patients. No doubt much of this can be ascribed to the fact that these patients were bed or chair ridden for many years and were poorly developed. The layers of the vessels were equally diminished. In 3 of the 4 patients there were intimal atheromatous deposits. They appeared rather extensive for patients of the age group of these patients.

Two of the 4 patients showed pneumonia due to aspiration of lipid. In 1 patient (case 3) it was extensive and severe. In the other (case 1) it was confined to a small portion of one lobe. Edema was present in areas uninvolved by pneumonia. Considering the lesions in the striated muscle of the esophagus, the pharynx and the diaphragm and in the intercostal muscles, it is remarkable that more extensive pneumonitis did not occur. No lesions were noted in the smooth muscle of the bronchi. Atelectasis and pulmonary edema occurred in the patient with generalized peritonitis. The patient (case 2) who showed extensive fatty replacement of the myocardium of the right ventricle, as well as a small

amount of fibrosis of the left ventricle, also had pulmonary edema.

Persistent thymus has been noted in progressive muscular dystrophy. Johnson[17] reported necropsies of 2 patients who were brothers. The ages at death were 17 and 18 years. Both died suddenly, a fact which Johnson considered unusual. Sudden death, however, has occurred in patients observed in this institution. He attributed their sudden death to noninvolution of the thymus and status lymphaticus but at the same time noted pleural and abdominal effusions, cardiac dilatation and fibrosis and fatty replacement of the myocardium. All this is suggestive of cardiac failure. Globus[8] reported subinvolution of the thymus in a patient aged 10. In only 1 of the patients whose cases are reported here was thymic tissue found. The amount of lymphoid tissue elsewhere did not appear excessive for persons in this age group.

Fatty change was noted in the liver of 1 patient. In the liver of another there were anomalous dilated blood channels with the structure of veins. This patient also showed ectopic pancreatic tissue.

The thyroid gland was examined in 3 cases and appeared to be normal. The pancreas and the pituitary and adrenal glands were also apparently normal. The Italian school has reported numerous abnormalities involving especially the pancreas[18] and the pituitary gland,[19] the occurrence of which, however, I have not been able to substantiate.

The only renal lesions found were recent infarcts and hemorrhage in the pelvis in case 1.

The amount and the condition of the smooth muscle in the prostate could not be intelligently appraised in the present connection because of normal variations in the fibrous tissue. The glandular tissue was normal except for early adenomatoid hyperplasia in 1 case.

The smooth muscle about the epididymides and the testes was edematous only in the patient who was in cardiac failure. There was active spermatogenesis in all 3 patients. Leydig cells were not numerous in any sections and, when present, contained only small amounts of pigment.

The muscularis of the blood vessels was normal in all sections. Several sections of the sympathetic trunks were examined in 2 cases, and nothing noteworthy was seen.

Bramwell[4] has commented on hypertrichosis, occurring especially below the knees. This was

16. Bevans, M.: Am. J. Path. **21**:25, 1945.

17. Johnson, W. J.: M. Rec. **144**:506, 1936.

18. Bompiani, G., and Medolesi, G.: Policlinico (sez. med.) **43**:593, 1936.

19. Franzolin, C.: Clin. med. ital. **68**:267, 1937.

noted in 1 of our 4 cases. It has been seen even more often in patients who are still under observation.

The brain was examined in 3 cases and was normal. Unfortunately, no permission was granted for examination of the spinal cord.

SUMMARY

Four patients with progressive muscular dystrophy came to necropsy. All presented myocardial lesions of varying severity. The lesions were similar to, but not identical with, those in the skeletal muscles. Although clinically only 1 patient was in heart failure, the other patients showed disturbances of cardiac rhythm.

The striated muscle of the tongue and the upper part of the esophagus showed changes which were milder than those observed in the skeletal muscles. There were no detectable clinical symptoms referable to these lesions except in 1 patient, who had difficulty in swallowing.

The smooth muscle of the gastrointestinal tract showed edema, variation in size, atrophy and disappearance of the smooth muscle cells and, occasionally, small areas of fibrosis. Gross lesions included marked dilatation of the stomach in 2 cases and perforation in 1, and dilatation of the colon in 2 cases with fecal impaction in 1. The changes in smooth muscle were not considered specific, although it is suggested that they were comparable in many ways to those seen in the skeletal muscle. The alterations described are thought to explain some of the clinical symptoms referable to the gastrointestinal tract.

No abnormalities of the endocrine glands were found except for the presence of thymic tissue in 1 case. No lesions of the smooth muscle of the vascular system or of other organs were noted.

STRUCTURE OF THE LIVER IN PELLAGRA

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Little is known about the pathologic reactions of the human liver in pellagra and other forms of malnutrition or the role of this organ in the genesis of these diseases. That some damage of the liver is present in pellagra has been mentioned frequently.[1] Such information has been derived chiefly from biochemical studies or postmortem observations. As we shall show in a later study, extensive damage of the liver may not be reflected in significant deviations of biochemical tests at all. Moreover, the comparison of the histologic appearance of the liver with the results of laboratory tests leaves little doubt in our minds that the present biochemical procedures are much too crude in many instances to assess even gross hepatic damage as seen microscopically. Information derived from postmortem specimens can also be misleading, if only because the factors responsible for death are so variable and so inconstant.

The intensely fatty liver described in the infantile pellagrin has for the most part been attributed to intercurrent infection affecting chiefly the bowel or the respiratory tract.[2] It was not until specimens were obtained by repeatedly puncturing the livers of children at various stages during the course of the disease that we were able to establish beyond doubt that the fatty liver is as constant a feature of the syndrome as the rash or the edema and that it develops rather early in the disease while this is still free from other known complications, such as pneumonia, tuberculosis, gastroenteritis and diphtheria.

The structural modifications in the human liver in pellagra are indeed remarkable. It is doubtful whether our knowledge would ever have been as complete as we shall indicate in this study without the liver puncture procedure. This method has more than justified itself, for in no other way has it been possible to study the liver in man in such a dynamic fashion. The very fact that the liver of the same patient has been studied throughout the acute stages of pellagra, not to mention the effect of repeated attacks of this disease, has provided a unique opportunity for examining in considerable detail the progress of the pathologic processes from the time the liver has become extremely fatty until the appearance of well marked pigmentary cirrhosis. That the fatty liver seen at postmortem examination of the pellagrin may result from dietary imbalance has been repeatedly suspected from the numerous experiments conducted on animals,[3] but we have been privileged to demonstrate that this type of liver is a constant feature in malnourished infants and that the degree of fatty change is a much better index of the prognosis than the clinical picture.[4c]

The liver biopsy technic has allowed us to state with assurance that cirrhosis of the liver in man can be produced by dietetic imbalance. Moreover, the causation of that puzzling disease hemochromatosis up to the present has baffled clinicians and pathologists alike. Even Sheldon[5] after his masterly review of the literature in the light of his own experience was forced to conclude that this disease is the result of an inborn error of metabolism. As far as our own investigations are concerned, after carrying out more than 400 biopsies on 120 patients we may state with assurance that hemochromatosis can be regarded as one of the commonest sequelae of pellagra.

It has been generally accepted that pellagra is caused by dietary imbalance, and by the same token we shall show that hemochromatosis is the result of very chronic malnutrition, which at various stages throughout the progress of the disease may be punctuated by clinical manifesta-

From the University of the Witwatersrand Medical School.

1. (a) Spies, T. D.; Sasaki, Y., and Cross, E.: South. M. J. 31:483,1938. (b) Slatineanu, A., and others: Compt. rend. Soc. de biol. 116:1113, 1934. (c) Sydenstricker, V. P., and others: Am. J. M. Sc. 197:755, 1939.

2. Trowell, H. C.: Tr. Roy. Soc. Trop. Med. & Hyg. 33:389, 1940.

3. (a) György, P.: Am. J. Clin. Path. 14:67, 1944. (b) Himsworth, H. P., and Glynn, L. E.: Lancet 1:457, 1944. (c) György, P., and Goldblatt, H.: J. Exper. Med. 75:355, 1942.

4. (a) Gillman, T.; Gillman, J.; Inglis, J.; Friedlander, L., and Hammar, E.: Nature, London 154:210, 1944. (b) Gillman, T., and Gillman, J.: J. A. M. A. 129:12, 1945; (c) Arch. Int. Med. 76:63, 1945.

5. Sheldon, J. H.: Haemochromatosis, London, Oxford University Press, 1935.

tions of acute malnutrition which may, for a time at least, partially obscure the underlying chronic lesion.

This study of the livers of 120 pellagrins examined on the day of admission to hospital has allowed us to classify the various lesions seen in these livers in such a way that it is now possible to obtain a dynamic expression of all the stages which we have shown to occur in the development of pigmentary cirrhosis. This classification has been materially facilitated and its validity upheld by repeated punctures of the liver of the same patient not only while the patient was undergoing treatment but also on later occasions when the patient was readmitted during a relapse.

Since every pellagrin admitted to hospital has not the same degree of hepatic damage, it is unreasonable to attempt to treat every patient in the same routine fashion. Hitherto, the efficacy of treatment has been judged exclusively by the healing of the external lesions and the subjective improvement. We are now convinced from this and other of our studies of the liver in malnutrition that these criteria can be extremely misleading and even dangerous, especially since the hepatic lesions in many pellagrins do not become arrested but in fact continue silently active despite the external improvement. With the failure to arrest or cure the pathologic process in the liver during the acute stages, the disease progresses in such a way that eventually the liver alters its pattern of reaction. Once the liver cells commence to deposit pigment in excessive amounts and cirrhosis develops, it is obvious that the character of the disease has altered. It is only with the aid of the liver puncture technic that we are able to assess the extent of the hepatic damage in each patient and thus base our therapy on the histologic observations rather than on the erratic and misleading clinical manifestations of the disease. The main objects of this study are, first, to draw attention to the serious damage of the liver induced by acute and chronic malnutrition; second, to demonstrate the changes in the reactivity of the liver at different ages; third, to provide satisfactory criteria for grading pellagrins according to the structure of the liver, and finally to record the stages in the development of pigmentary cirrhosis and to demonstrate that this lesion is one of the end results of chronic malnutrition.

MATERIAL AND METHODS

This report is based on a study of specimens of liver removed by a modified aspiration biopsy method,[6] from 120 pellagrins on the day of admission to hospital.

6. The method is described in the South African Journal of Medical Science (**10**:53, 1945).

The clinical manifestations of malnutrition observed in infants and children have been fully described in a previous publication.[4] The diagnosis of pellagra in adolescents and adults was made on the basis of the typical dermatitis, glossitis, diarrhea and dementia as well as other external evidences of avitaminosis. A full analysis of the clinical features observed in adult pellagrins will be presented shortly, but at this stage it may be said that all of the patients manifested one or more signs of severe, active pellagra and that frequently obvious evidence of the syndrome of ariboflavinosis described by György and others [7] was also present.

Immediately after the core of liver tissue had been aspirated, it was divided into two or more portions and fixed. The routine fixatives were a 4 per cent solution of formaldehyde and Helly's fluid plus osmic acid, but tissues from a large number of our patients were fixed in Bouin's fluid, alcoholic trinitrophenol-formaldehyde solution, 85 per cent alcohol and 5 per cent formaldehyde-saline solution in order to prepare sections for detailed microchemical studies.

Detailed investigations have already been made on mitochondria, fat, glycogen, acid and alkaline phosphatases, fluorescent material, oxidase-containing material, vitamin A (by fluoroscope), iron and several other intracellular organoids and enzymes. However, this report is based largely on frozen sections stained with sudan IV and hematoxylin and preparations stained for iron.

We have found during the course of this study that routine hematoxylin and eosin staining of paraffin sections is of limited value, and we consider that considerably more can be learned from the study of frozen sections stained with sudan IV and counterstained with hematoxylin.

Much of the brown to black pigment commonly encountered in liver cells failed to give a positive iron reaction with the standard ferrocyanide tests. A modification of Perl's method was elaborated which is highly selective and at the same time simple and rapid. According to this modified technic, sections are covered with a mixture of equal parts of 2 per cent potassium ferrocyanide and 5 per cent hydrochloric acid in 70 per cent alcohol. The mixture is made fresh and heated to boiling for use. When the pigment in the liver cells is black, it is advisable to heat the acid-ferrocyanide solution during staining. Sections were treated with this solution for one to two minutes, washed and counterstained. When paraffin sections fixed in alcohol or in solution of formaldehyde were being prepared, they were counterstained for three to five minutes with a 1: 6,000 aqueous solution of basic fuchsin. However, beautiful and valuable preparations can be made by treating frozen sections with hot alcoholic acid-ferrocyanide solution and then, after washing in distilled water, counterstaining with sudan IV and hematoxylin. This method was indispensable in this study since it was the only technic whereby it was possible to examine the relationship between intracellular fat, hemofuscin and iron pigment.

While this report is primarily concerned with the pathologic changes occurring in the liver in pellagrins on the day of admission to hospital, we have nevertheless used certain information gleaned from the study of the changes produced in these same livers by various forms of therapy in order to elucidate certain aspects of the genesis of the hepatic lesions. In all, more than 600 biopsies have been performed on pellagrins before, during and after

7. György, P., in Evans, E. A., Jr.: The Biological Action of the Vitamins, Chicago, University of Chicago Press, 1942, p. 54.

therapy, and the evidence available from all this material has been taken into account in presenting this report.

CLASSIFICATION OF LIVERS

The structure of the livers of pellagrins as revealed by biopsy specimens can be so divergent on admission of the patients to the hospital that with a small number of cases it would be impossible to establish any consistent pathologic process. This can be readily appreciated from a glance at figures 2 and 11 representing 2 different cases of pellagra. In figure 2 the cells are loaded with large globules of fat, while in figure 11 the outstanding feature is the presence of masses of iron-containing granular pigment. It would be scarcely justifiable to regard these two conditions as stages in a common pathologic process. However, after examination of the livers of 120 pellagrins before and after treatment it has been possible to trace the genesis of the hepatic lesion in such a way that these two extreme alterations may be regarded as manifestations of a single process which may culminate in pigmentary cirrhosis.

In order to facilitate description and at the same time to provide reliable criteria for assessing the degree of hepatic injury seen in a patient at a particular stage in the evolution of the disease, we have suggested a classification of the livers of pellagrins based on the presence of the fat, the amount and the distribution of the pigment and the presence or the absence of cirrhosis.

Type 1 includes all those livers in which the accumulation of fat in the absence of hepatic cellular pigment and cirrhosis is the main feature.

Type 2 includes those characterized by the presence of iron-containing pigment chiefly in the hepatic cells and unassociated with cirrhosis. In many of these livers there may be a superimposed fatty change of varying intensity as in type 1.

Type 3 is similar to type 2 except that the iron-containing pigment is aggregated in large masses in liver cells, in Kupffer cells or in cells lying in the portal tracts.

Type 4 includes all the livers unmistakably cirrhotic, the cirrhosis in every instance being associated with masses of iron-containing pigment.

Any classification of this sort is open to obvious objections, chief of which is that it tends to give a static concept to a highly dynamic process. The second, but not less serious, is the tendency to regard a type as a sharply defined entity. Despite these objections, however, livers do show some structure at a moment in time and in order to provide the landmarks necessary both for grading the livers and for assessing any therapeutic measures in terms of the pathologic reaction in the liver, such a classification is fully justified.

The main features of the four types of livers will be considered seriatim.

TYPE 1 (FIGS. 1 TO 6)

The histologic appearance of type 1 has been described elsewhere,[4] but it is necessary to outline briefly the main features and to extend this description to include the fatty livers seen in adults. Using as criteria the amount, the distribution and the physical appearance of the fat in sudan IV–stained preparations, we have subdivided type 1 into six subtypes (table).

Type 1a (fig. 1).—In the first subtype the fat is distributed diffusely throughout the entire lobule. Almost every cell is crowded with fine droplets more or less of the same size, being about a third to half the size of the nucleus (fig. 1). Those cells which contain only a few droplets usually exhibit a clear vacuolated cytoplasm.

The sharply delimited cell walls appear to bulge outward into the sinusoids or laterally, in which case they compress adjacent hepatic cells. The sinusoids are usually much narrowed. Occasionally, livers of this subtype may contain large globules of fat about the size of the nucleus, but the presence of the finer droplets in the overwhelming number of cells is sufficient grounds for excluding these livers from the next closely related subtype (1 b.).

Type 1b (fig. 2).—The outstanding feature of these livers was the great abundance of fat distributed throughout the tissue. The fat was in the form of a single large pale-staining globule filling the entire cell. The nucleus was pushed to one pole, and the cytoplasm was reduced to a narrow rim compressed against the cell membrane. Almost every single cell was involved in this severe fatty change. In fact, the livers were so fatty that they floated in the fixative. There was no necrosis or hemorrhage. Actually, the liver was remarkably avascular. Despite the relatively large pieces of liver available, it was extremely difficult to identify any but the larger radicles of the hepatic veins. The sinusoids were closed, but their position was indicated by rows of flattened Kupffer cells, which remained fat free except on rare occasions when only a small droplet was observed. The portal tracts were difficult to identify because of the great enlargement of the lobule caused by the massive concentration of fat in the individual cells. In some instances a moderate accumulation of round cells was present in the portal tracts. The reticulum was not thickened. In general, the histologic appearance of this type of liver was such that the organ could be mistaken for perirenal fat.

Livers of type 1 b do not resemble in any way the livers described in cases of acute or chronic carbon tetrachloride poisoning, but they do have a striking resemblance to the livers of rats fed for two hundred days on a diet of "mealie pap" (corn meal porridge) and sour milk.[8] Type 1 b is the type of hepatic structure encountered in children who die from infantile pellagra.[9]

Type 1c (fig. 3).—While the cells throughout the major portion of the lobule contain numerous small droplets of fat, in the cells immediately related to the portal tracts the fat is in the form of a single large globule similar to that characteristic of livers of type 1 b. Many of the larger homogeneous globules appear to lie in vacuoles, the wall of the vacuole being usually larger than the fat droplet. Frequently round cells accumulate in the portal tracts, which in turn become so accentuated that they can easily be mistaken for the sclerosis seen in cirrhosis. These cells come and go in a seemingly rhythmic fashion and may be abundant at the time the fat is being absorbed.

Type 1d (fig. 4).—The amount of fat present, although still abundant, is very much less than that in livers belonging to type 1 c. At least two or three layers of cells around the radicles of the hepatic vein, are almost devoid of fat. In the midzone of the lobule, the discrete fat droplets stain intensely with sudan IV. These droplets may be of all sizes; some are dustlike orange-red particles scattered throughout the cytoplasm, while others are globules so large that they exceed the size of the nuclei. These large globules are distributed chiefly near the portal tracts, and they lie in a distinct vacuole, the wall of which is sharply delimited from the rest of the cytoplasm. In some cells there may

8. Gillman, J.; Gillman, T.; Gilbert, C., and Mandelstam, J.: Brit. J. Exper. Path. **26**:67 ,1945.

9. Strachan, A. S., cited by Sheldon,[8] p. 5. Gillman and others.[4]
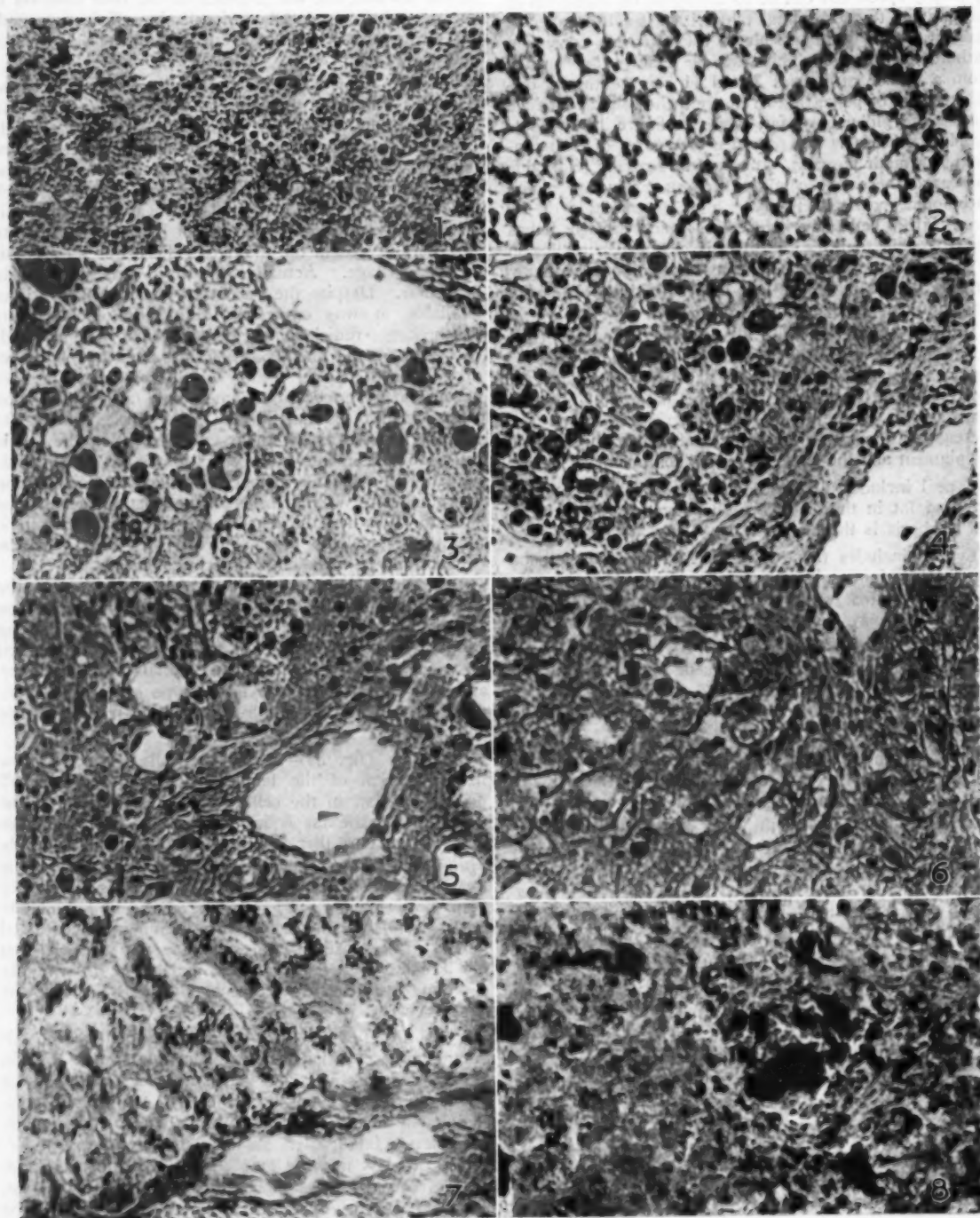
(See legends on opposite page)

be one intensely staining globule half as large again as the nucleus, with several smaller globules lying in the peripheral region of the cytoplasm. Intermingled with these cells containing large globules of fat are a few cells with water-clear cytoplasm and ballooned cell walls.

The portal tracts are flanked by two or three rows of these enlarged fat-containing cells or large vacuolated cells rimmed with brightly staining lipoid. For this reason the portal tracts are prominently outlined, and this arrangement is a distinctive feature of livers belonging to the type 1 d series. It might be mentioned at this stage that many clear round areas are frequently visible within the fat globules. At a later stage, these small vacuoles increase in size, and a large fat globule is replaced by a large clear vacuole coated with a thin film of lipoid (fig. 14). Frequently these vacuolated areas can be mistaken for fat-containing cells in which the fat globule has fallen out, an event which is not uncommon during the preparation of sections in which the liver cells are filled with large fat globules as in the type 1 b series. Such large vacuoles in the liver cells, as will be indicated later, represent a stage in the absorption of the fat.

The sinusoids are for the most part closed, but here and there a long segment may be seen to be dilated. The portal tracts are easily identified, first, because of the localization of the large fat-containing and vacuolated hepatic cells and, second, because there is a moderate accumulation of round cells in the connective tissue. Lymphocytes are less numerous in the smaller radicles of the portal tract.

Type 1 e (fig. 5).—The livers are in general similar to those belonging to type 1 d except that there is much less fat in the central and midzonal regions of the lobule. The fat is so constantly localized to the periportal regions that the outlines of the lobules are accurately demonstrated. The fat is predominantly in the form of small globules, although some may be about twice the diameter of the nucleus; all gradations exist between these and the smallest dustlike particles. The globules are stained intensely with sudan IV; in fact, they look like round masses of colloid. Often the larger globules appear to be riddled with vacuoles, which may coalesce to give a moth-eaten appearance to the large globules. However, all shades of colors from deep orange red to lemon yellow may be seen in the fat globules stained with sudan IV. The pale-staining fat is found as a rule in livers in which the fat is known to be disappearing slowly. The presence

of colloid-like globules of fat in association with vacuoles is also an indication of the slow absorption of fat, as was confirmed time and again by repeated biopsies at weekly intervals in the same patients recovering from pellagra, whose livers initially belonged to type 1 b.

The sinusoids near the portal tracts can be distinguished only with difficulty. Actually their position is indicated only by the rows of Kupffer cells. Toward the center of the lobule, however, the sinusoids are easily identified as open channels skirted by Kupffer cells filled with discrete globules of fat. As in the previous two subtypes, moderate numbers of round cells are frequently concentrated in the larger portal tracts.

Type 1 f (fig. 6).—Livers in this category would quite erroneously be regarded as normal. Some of the liver cells are grossly enlarged, the cytoplasm may be vacuolated, and the fat is scanty but is in the form of densely stained small globules unevenly distributed throughout the glandular tissue. Under treatment this fat is progressively absorbed, and on discharge only the Kupffer cells contain fat. For the rest there is no other detectable abnormality.

TYPE 2 (FIG. 7)

The presence of hepatocellular iron pigment and hemofuscin is the distinctive characteristic of livers of type 2. This pigment is essentially in the form of moderately fine irregular granules and is restricted at first to the biliary pole of the cell (fig. 16). We have strong reason to suspect that when the pigment is found in the majority of the liver cells throughout the entire lobule, clumping and massing of pigment in the portal tracts, a feature of type 3 livers, frequently occur, provided a sufficiently large piece of tissue is available for examination. As biopsy specimens are of necessity relatively small, it is not an unusual experience to find that a liver is extensively pigmented and still shows no evidence of massive aggregation of pigment. On the other hand, we have seen large sections of livers, obtained at autopsy, in which the pigment was observed only in the form of granules localized to one pole of the cell. Therefore, it is necessary to establish a distinct class to include livers in which the pigment granules are discrete and localized in the liver cells.

In sudan IV–stained preparations, numerous round or angular yellow or yellowish brown particles are

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EXPLANATION OF FIGURES 1 TO 8

Fig. 1.—Liver, type 1 a. Multiple fat droplets are seen in the liver cells throughout the lobule. Frozen section stained with sudan IV and hematoxylin; × 250.

Fig. 2.—Liver, type 1 b. Every liver cell is filled with a single large fat globule. Frozen section; × 250.

Fig. 3.—Liver, type 1 c. Large colloid-like fat globules are concentrated in the cells around the portal tracts. Note also the large vacuolated cells. Frozen section; × 250.

Fig. 4.—Liver, type 1 d. Irregular-sized fat droplets extend from the portal tracts to the midzone. Frozen section; × 250.

Fig. 5.—Liver, type 1 e. Note small colloid-like droplets restricted to cells around the portal tracts and a few large vacuolated cells lined with residual fat. Frozen section; × 250.

Fig. 6.—Liver, type 1 f. Occasional colloid-like fat droplets and some vacuolated cells are scattered irregularly through the lobule. Frozen section; × 250.

Fig. 7.—Liver, type 2. Note that the iron is localized to the hepatic cells, with no evidence of iron in the portal tract. Paraffin section to demonstrate iron; × 250.

Fig. 8.—Early type 3 liver showing aggregations of iron pigment in the portal tract as well as in the hepatic and Kupffer cells. Paraffin section to demonstrate iron; × 250.
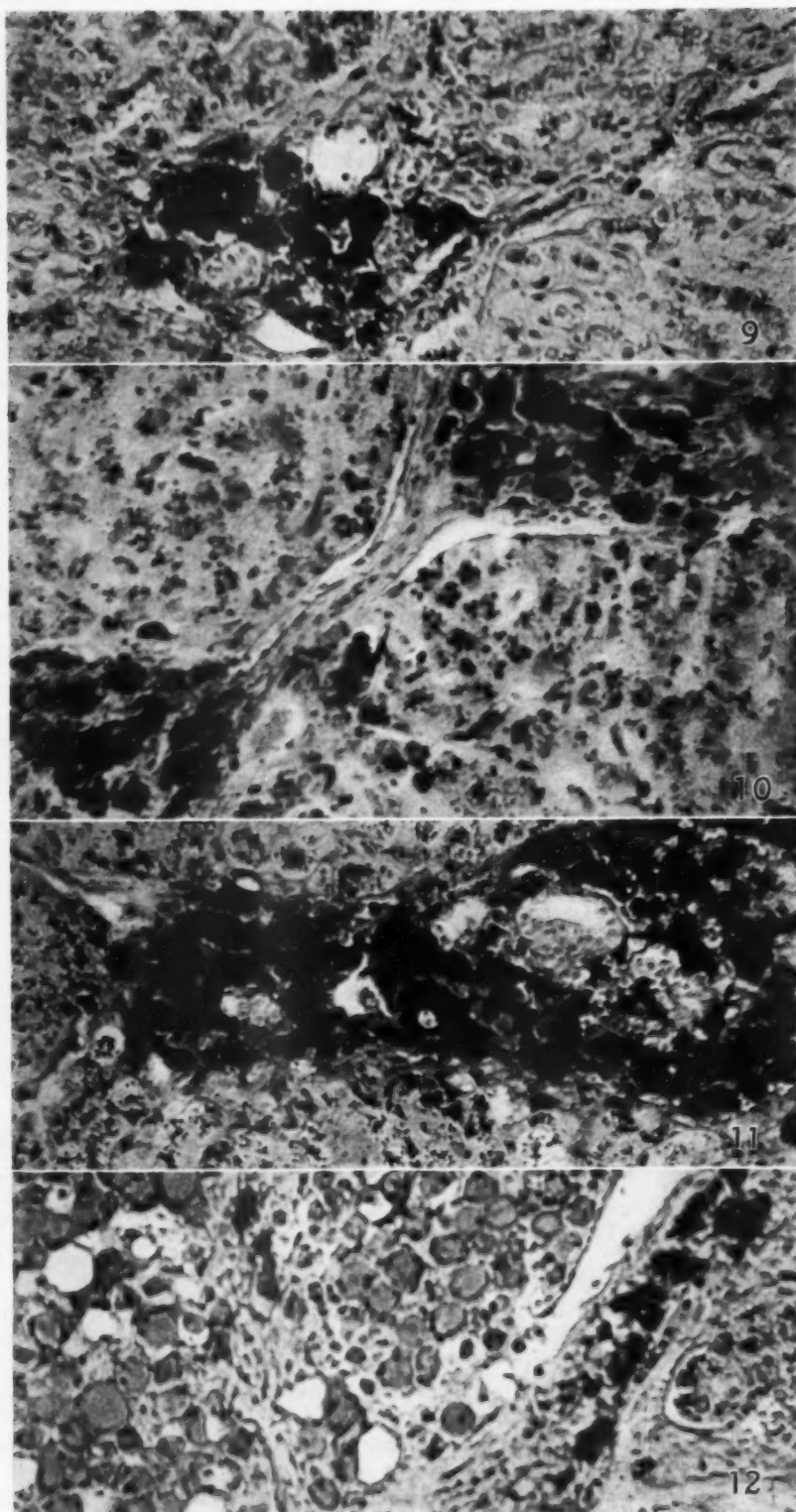
(See legends on opposite page)

seen lying in almost all the liver cells. They may be so numerous as to form a granular mass occupying the whole of the area above the nucleus. All gradations in color between golden yellow and dark brown or even black can be recognized, especially in cells lodged near the portal tract. In many instances, the outer rim of the particle is deep brown or black while the inner zone is stained yellow. When the pigment is abundant, the localization is at first so precise that the distribution of the intralobular bile capillaries is accurately mapped out. In many livers the cells in the central and midzonal regions of the lobules do not contain much pigment, but around the portal tracts the liver cells are so intensely pigmented that under very low power the position of the larger and smaller portal tracts can be readily identified by the concentration of pigment in the adjacent hepatic cells. Although sooner or later the majority of liver cells may become pigmented, those situated in the center of the lobule usually are first affected; the periportally situated cells are much more intensely pigmented, and the pigment remains there for a much longer time. The Kupffer cells contain fat globules, but the yellow and brown particles are often scattered amidst the lipoidal globules and occasionally transitional stages exist in these phagocytes between the yellow and the dark brown or black granules.

In sections prepared from paraffin blocks and treated with our modified technic for iron and counterstained with a weak solution of basic fuchsin, it is evident that all the dark brown and black particles are in fact iron pigment. Amidst the blue-stained iron pigment, red or reddish brown particles are also present. These are identical in position, size and shape with the yellow granules demonstrated in sudan IV–stained preparations. This pigment has all the features attributed to hemofuscin. The hemofuscin is often coated with an irregular layer of blue-staining iron. By a combination of technics whereby the fat and the iron pigment are stained simultaneously in a frozen section it was shown that hemofuscin, which stains faintly with sudan IV, may also be rimmed with blue-stained iron-containing material (fig. 16). With this combined technic we have not been able to identify any other pigments except those which reacted positively for iron or fat. However, we are of the opinion that both the iron and the fat pigments contain a considerable amount of protein. The hemofuscin and the hemosiderin make their appearance simultaneously, first in the biliary pole of the cell.

Although in this type of liver the iron pigment and the hemofuscin are found chiefly in hepatic cells, they are frequently seen also in the Kupffer cells. However, on numerous occasions, despite the widespread distribution of pigment in the hepatic cells, Kupffer cells contained only fat and no pigment (fig. 22).

The pigmented cells in the type 2 livers (fig. 19) do not lose their capacity to form fat. Actually any variety of fat described for the type 1 livers may be encountered. Only when the liver cell is crowded with iron and all the mitochondria have disappeared does it lose its capacity to form fat. Thus a type 2 liver in which the cells are filled with fine droplets of fat is classified as type 2 a. It is similar in all respects to type 1 a except that in addition to the fat there are also demonstrable intracellular iron and hemofuscin. In this manner the type 2 liver may be graded as type 2 a to f depending on the distribution and the size of the fat droplets.

When the cell contains fat and pigment, the position of the latter is determined largely by the amount and the physical form of the fat. When the fat is distributed as fine droplets the localization of the pigment between the nucleus and the biliary pole is not grossly disturbed (fig. 19), but when the large single fat globule is present, it may be difficult at first to identify the pigment, which may be pushed against the cell membrane (figs. 20 and 21). Sometimes in such instances, too, the pigment is found in the small area of cytoplasm in which the nucleus is lodged. However, in sections stained to demonstrate simultaneously the fat and the iron the presence or the absence of pigment can be readily ascertained (fig. 21). At a certain stage, when the cell is crowded with pigment, it apparently is no longer able to form fat (fig. 18).

TYPE 3 (FIGS. 8 AND 9)

The characteristic feature of livers of type 3 is the massive aggregation of iron pigment in hepatic and Kupffer cells scattered irregularly throughout the lobule (figs. 8 and 9). These clumps of pigment are especially noticeable in the portal tracts, where as a rule they are lodged in the histiocytes. The pigment may be so abundant that in the iron-stained preparations the portal tracts are outlined by the blue-stained masses of iron. In order to avoid any confusion we have insisted that any clumping of iron in the liver cells, the stellate cells or the portal tracts should be sufficient indication to include such livers in the category of type 3. In advanced pellagra almost all the liver cells contain some iron pigment, but often the pigment is concentrated, especially in the periportal zone, while the cells near the central vein may exhibit only a few granules of iron pigment. The pigment in the cells near the portal tracts is still arranged near the biliary pole of the cell, but it is much coarser than in livers of type 2 (compare figs. 16 and 18). The Kupffer cells also contain large masses of pigment, but many may be devoid of pigment. The liver cells filled with pigment degenerate very slowly, and it is likely that after they are destroyed the liberated particles of pigment are ingested by nearby stellate cells. The thickened portal tracts are much more prominent than in livers of type 2 (fig. 9). This is due to the masses of round cells and some fibroblastic elements in addition to the heavy deposits of pigment. Approximately 45 per cent of the specimens belonging to type 3

EXPLANATION OF FIGURES 9 TO 12

Fig. 9.—Late type 3 liver showing large aggregations of iron pigment in moderately thickened portal tracts and heavy concentrations in the liver cells, especially near the portal tract. Paraffin section to demonstrate iron; × 228.5.

Fig. 10.—Early type 4 liver. Note cirrhosis and heavy pigment in the portal tracts as well as in the adjacent liver cells. Paraffin section to demonstrate iron; × 228.5.

Fig. 11.—Liver, type 4. Well marked pigmentary cirrhosis. Paraffin section to demonstrate iron; × 228.5.

Fig. 12.—Early type 4 b. Note thickened, pigmented portal tracts with liver cells filled with single large fat globules as in type 1 b. Frozen section stained for fat and iron; × 228.5.
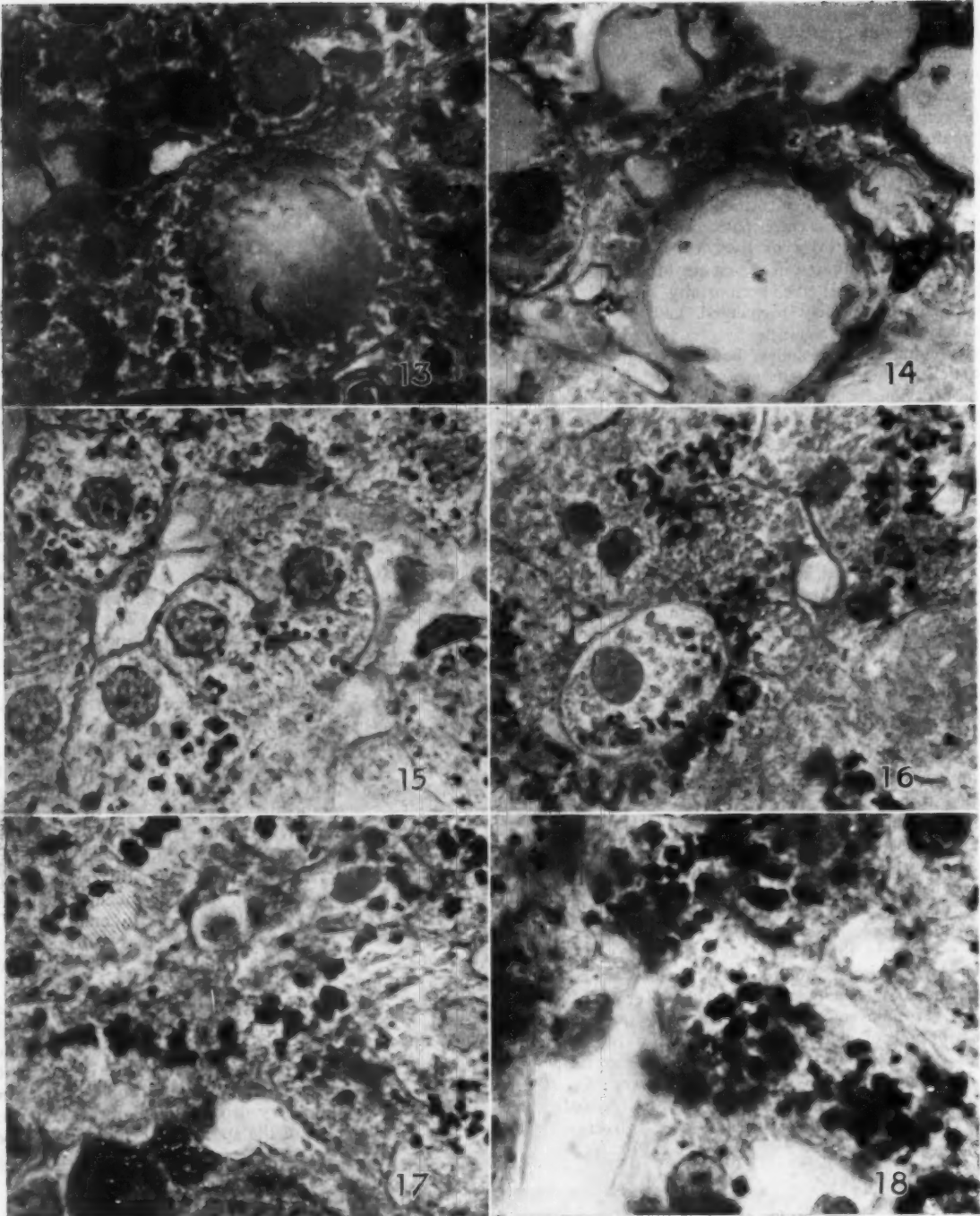
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could easily be graded as cirrhotic (type 4) if less exacting criteria for cirrhosis were used. In type 3 there are transitions between type 2 and type 4.

As in type 2, fatty change occurs frequently, and the livers may be graded according to their fat content.

TYPE 4 (FIGS. 10, 11 AND 12)

The dominant feature of livers of type 4 is the presence of pigmentary cirrhosis in a form which cannot be disputed histologically (figs. 10, 11 and 12). Macroscopically, the fragments of liver usually have an intense rusty brown color alternating with pale yellowish bands of tissue.

The pigment as a rule is much heavier in the region related to the portal tracts. In some cases, however, the iron is uniformly distributed throughout the lobule. Irrespective of the distribution of the pigment, the large portal tracts are invariably thickened, and this thickening is exaggerated by masses of coarse, irregularly clumped dark brown pigment.

In preparations to demonstrate iron the portal tracts are filled with blue-green masses. The iron appears to be enclosed in much enlarged cells which are grouped around the constituent portions of the tract. The lymphatic vessels are colored deep blue, and the walls of the artery and of the portal vein also show the presence of iron. The iron pigment outlines the lobule clearly, and even the smallest radicles of the portal tract are noticeably thickened and contain clumped masses of iron pigment.

In the earliest stages the cirrhosis is of the monolobular variety, the structure of the lobule being well preserved. Only the reticulum of the sinusoids nearest the portal tract is prominent. There is no profound alteration in the vascularity of the sinusoids as described by Connor [10] in alcoholic cirrhosis. In advanced cirrhosis bile duct proliferation is not common, although in 3 instances the ductules seemed to be more numerous than usual. In 1 case there appeared to be distinct hyperplasia of bile ducts. In advanced disease the fibrous tissue overgrowth is so great and the degeneration of the liver cells so marked that the hepatic lobules seem to be half their normal size. The overgrowth of connective tissue is accomplished rather slowly, and the soft cellular fibrovascular tissue seen in cirrhotic livers produced experimentally in animals was not a feature in our hemochromatotic livers. Round cell infiltration of the portal tract at some stages may be intense and the cirrhosis appears to be much greater than is actually the case. The reduction in the number of round cells seen in a fragment of tissue removed from the same patient at a later stage emphasizes once again that these round cells may come and go in a mysterious fashion. These cells cannot be regarded as a precursor of cirrhosis, especially since they are often found in livers regarded histologically as normal.

In many areas the pigment in the liver cells is still restricted only to the biliary pole, but in others this localization is completely lost, as the pigment is so abundant that it crowds the cell and occupies almost every available space in the cytoplasm (figs. 11 and 18). Such heavily pigmented cells may occur in groups (figs. 18 and 19). It is sometimes possible to mistake a pigmented liver cell for a Kupffer cell, because it often becomes detached from the other cells of the trabecula. If these pigmented human livers are allowed to autolyze, the pigment is further decomposed and there may be a blue-green flush throughout the cytoplasm. This feature is particularly noticeable where the pigment only half fills the cells. Such cells are commonly encountered in livers removed twenty-four hours after the patients have died. The greenish blue flush cannot be regarded as depositing pigment, for when the pigment does deposit it is always present, even from the earliest appearance, as discrete fine granules (figs. 15 and 16). However, on occasions when we have had reason to suspect that the iron pigment is absorbing, a greenish blue flush pervaded the cytoplasm. This is demonstrated especially well in alcohol-fixed tissue.

The Kupffer cells, too, are choked with masses of pigment, but like some liver cells they may contain only a few granules of iron even when the liver is grossly pigmented and cirrhotic. The cells lying in the sinusoids near the thickened tracts are most heavily pigmented. Sometimes a cell may be so crowded with an assemblage of pigment that it is impossible to imagine how it preserves sufficient vitality to adhere to the reticulum of the sinusoid or even to maintain its integrity sufficiently well to be identified as a Kupffer cell. Much of the pigment derived from degenerated hepatic and Kupffer cells apparently finds its way into the lymphatic vessels. There is some evidence to suggest that the iron pigment is carried along the lymphatics to the porta of the liver and thus to the upper abdominal lymph glands. The larger portal tracts are invariably much more pigmented than are the smaller ones.

It is often forgotten that the lymphatics of the liver arise in the liver tissue and drain via the porta hepatis. It seems to us that the pigmentation of the lymph glands of the upper abdominal group in hemochromatosis is due in large measure to the fact that these drain the liver as well as other related organs. To be sure, in extensive hemochromatosis, in which the pancreas is also commonly involved, the pigment may

10. Connor, C. L.: Am. J. Path. **14**:347, 1938.

EXPLANATION OF FIGURES 13 TO 18

Fig. 13.—Cell containing a single large fat globule with neighboring cells forming fat. Frozen section; × 1,100.

Fig. 14.—Fat absorbing with the formation of a single large fat-free vacuole rimmed by residual intensely staining fat. Frozen section; × 1,100.

Fig. 15.—Early pigment formation in hepatic cells of a type 2 liver. Frozen section stained for fat and iron; × 1,100.

Fig. 16.—Cells in a liver of type 2 with fine granules of cytosiderin (black) intermingled with cytolipochrome (gray) localized to the biliary pole. Frozen section stained for fat and iron; × 1,100.

Fig. 17.—Type 3 liver with coarse, irregular granules of iron pigment and heavy pigmentation of cells in the portal tract. Frozen section stained for fat and iron; × 1,100.

Fig. 18.—Massive accumulation of coarse, irregular clumps of iron-containing pigment in liver cells. Frozen section stained for fat and iron; × 1,100.
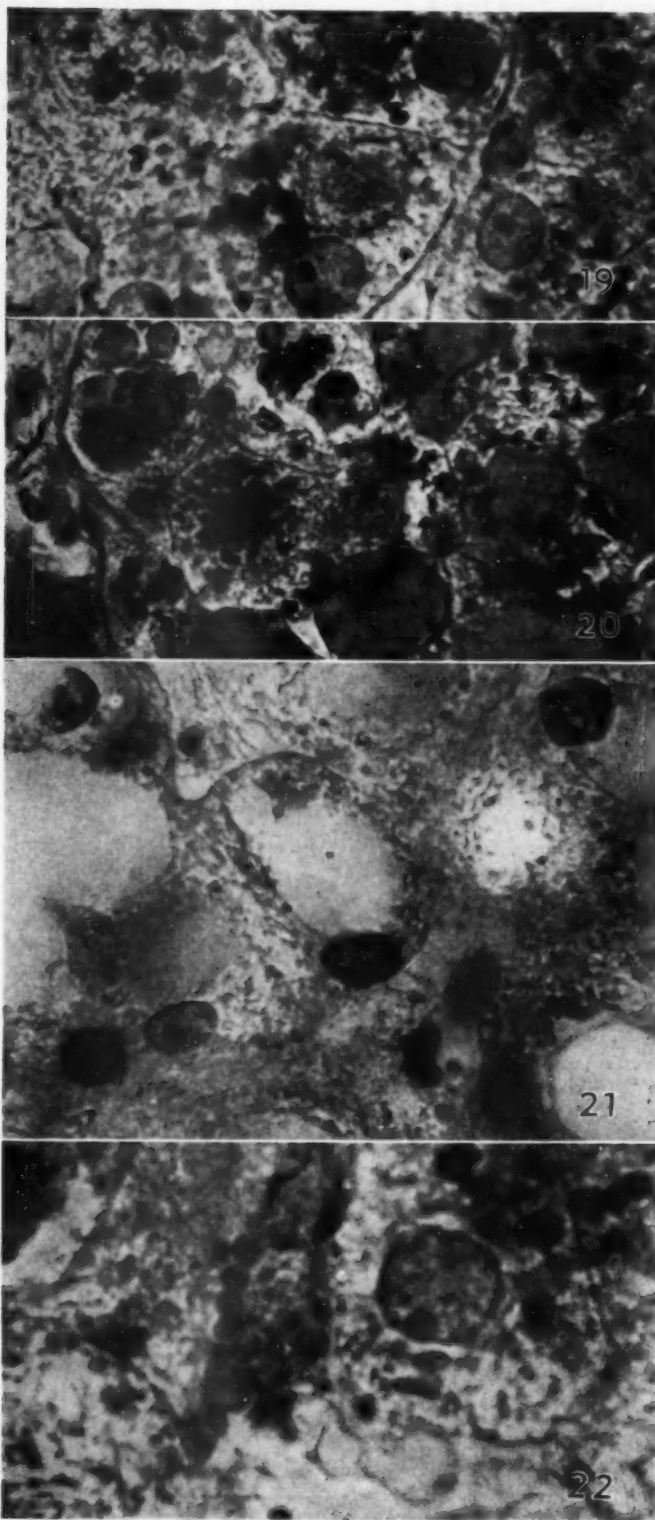
(See legends on opposite page)

also be derived from that gland, but there seems little doubt that the liver provides much of the pigment frequently observed in this group of lymph glands.

Those liver cells which are crowded to capacity with coarse pigment may be unable to form fat, but it is surprising to find that a heavily pigmented cell is still able to form large droplets of fat. Even in extensively cirrhotic livers the fatty change is still commonly found. The fat may appear in any of the forms described for the livers of type 1 (fig. 12). For this reason, it has been possible to grade livers of type 4 according to the nature and the distribution of fat.

abundant in those between the second and the fourth decade of life. It is extremely rare in infants and children, having been encountered in only 1 of 27 patients below the age of 10 years. However, in pellagrins past the age of 20 there is a sharp increase in the incidence of pigmented livers. In view of this peculiar age incidence of hepatocellular pigment, it is to be expected that all the livers of the pellagrous infants and

Histologic Classification of the Livers of One Hundred and Twenty Pellagrins on Admission to Hospital

	Type 1 (Fat Only)				Type 2 (Hepatocellular Pigment)						Type 3 (Hepatocellular Pigment and Clumps of Pigment in Portal Tracts)					Type 4 (Pigmentary Cirrhosis)							Totals		
Decade of life / Nature and Distribution of Fat	1	2	3	4	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	5	6	Sex	Males	Females	Total
No fat	0	0	0	0	0	0	5	1	0	1	0	0	4	0	1	0	0	2	0	0	0	Male	14		
	0	0	0	0	0	0	2	2	1	0	0	0	0	1	0	0	0	0	1	0	0	Female		7	21
(a) Diffuse fine fat droplets	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Male	3		
	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	Female		3	6
(b) Diffuse large fat globules	9	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	Male	12		
	7	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	Female		10	22
(c) Diffuse fine fat droplets and large globules around portal tracts	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	Male	5		
	6	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	Female		12	17
(d) Large vacuolated cells with colloid-drops near portal tracts	0	0	1	0	0	0	3	1	0	0	0	0	1	2	0	0	0	0	0	0	0	Male	8		
	0	0	1	2	0	0	2	0	1	0	0	0	1	1	0	0	0	0	0	0	0	Female		8	16
(e) Smaller colloid-like fat droplets near portal tracts	0	0	0	0	0	0	2	1	1	0	0	0	4	2	1	0	0	0	1	0	0	Male	12		
	0	0	0	0	1	0	3	1	1	0	0	0	1	2	0	0	0	2	0	0	0	Female		11	23
(f) Occasional fine droplets of fat	2	1	0	0	0	1	0	1	0	0	0	0	1	2	0	0	0	1	0	0	0	Male	9		
	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	1	0	0	Female		6	15
Total	13	2	1	1	0	2	12	5	1	1	0	0	11	6	2	0	0	4	2	0	0	Male	63		
	14	0	3	3	1	0	8	4	4	0	0	0	5	5	1	0	0	6	3	0	0	Female		57	
	27	3	4	4	1	2	20	9	5	1	0	0	16	11	3	0	0	10	5	0	0				120

Thus, in these livers fat may be graded from a to f according to its resemblance to the subtypes of the type 1 series.

AGE AND SEX INCIDENCE OF TYPES 1 TO 4

Before considering the sequence in the pathologic process seen in the livers of pellagrins it is necessary briefly to examine the age and sex incidence of the various types of diseased livers described. The findings in 120 cases are summarized in the accompanying table.

Hepatocellular pigment is a feature of pellagrins over the age of 10 years, being most children with a single exception should fall into the category of type 1. The nonpigmented, very fatty liver is typical of the very young pellagrin, with a preponderance of subtypes 1b and 1c. Not all adolescent and adult pellagrins, however, have pigmented livers, as 10 of the 37 livers of type 1 were seen in patients over the age of 10, and 8 of these patients were over the age of 20.

As previously indicated, livers with varying degrees of pigment may also contain varying amounts of histologically demonstrable fat. Actually only 17.5 per cent of adult pellagrins

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EXPLANATION OF FIGURES 19 TO 22

Fig. 19.—Type 2 a liver with different-sized droplets of fat lying in cells containing iron pigment. In one cell the fat globule is slightly larger than the nucleus. Frozen section stained for fat and iron; × 1,005.5.

Fig. 20.—Type 3 b liver. The majority of cells contain large single globules of fat; others contain multiple smaller globules in addition to the pigment. Frozen section stained for fat and iron; × 1,005.5.

Fig. 21.—Type 2 c liver. Note vacuolated large fat cells. In one cell the nucleus and the cytoplasm containing granules of iron pigment are compressed against the cell membrane. The vacuole is rimmed by densely staining fat. Note marked enlargement of liver cells. Frozen section stained for fat and iron; × 1,005.5.

Fig. 22.—Type 2 liver cell with iron pigment localized to the biliary pole. Note that the enlarged Kupffer cell is filled with uniformly sized fat globules and does not yet contain any iron pigment. Frozen section stained for fat and iron; × 2,011.5.

with pigmented livers were found to have no fat in their livers, whereas 82.5 per cent of all pellagrins had some fat in their livers. Whereas in infants and children the fat is chiefly of the variety described as subtype 1 b or subtype 1 c, in adults, when it is present, it is usually classified as subtypes a, e and f of type 1.

Pigmentary cirrhosis was found in 15 pellagrins (12.5 per cent), but at least 15 of the type 3 livers were obviously precirrhotic if not actually cirrhotic. It may be stated with reasonable certainty that in this series at least 20 per cent of all pellagrins showed positive evidence of incipient or frank cirrhosis. If we exclude the age group under 10 years, we find that among pellagrins seen at the Johannesburg General Hospital no less than 30 per cent bear the imprint of serious hepatic injury amounting to cirrhosis. In 2 children not included in the table there was irrefutable evidence of nonpigmented portal cirrhosis. Frank cirrhosis is seen chiefly in pellagrins under the age of 40 years. Pellagra in South Africa must be regarded as a serious disease, associated with a high incidence of pigmentary cirrhosis.

As far as the sex distribution of the various lesions in pellagra is concerned, it is evident from the table that this disease affects both sexes with equal frequency. This applies to adults as well as to children. Pigmentary cirrhosis, too, has no special election for either sex.

THE GENESIS OF THE HEPATIC LESION IN PELLAGRA

The classification of the livers of 120 pellagrins before treatment not only reflects the nature of the pathologic process, whether acute or chronic, but also represents the main stages in the evolution of pigmentary cirrhosis.

The fatty liver (type 1) in our cases is one of the manifestations of acute pellagra. Although this type of liver occurs in malnourished children, it is by no means restricted to the period of childhood, as it has been encountered in adolescents and in young adults during their first attack of pellagra and in alcoholic pellagrins. In both children and adults there is the usual history of a sudden change over from a rather good diet to an inferior one. The breast is obviously the best source of food for the baby. The African mother has a tendency to suckle the baby for a much longer period than the white mother, and when the baby is weaned, mealie pap becomes the staple of the diet. The sudden change over from breast milk to this inadequate diet results in an acute attack of malnutrition, as we may testify from the numerous cases of malnutrition encountered at the Johannesburg General Hospital over the last four years. In West Africa the name applied to this form of malignant malnutrition, "kwashiorkor," introduced by Williams,[11] actually "indicates the disease the deposed baby gets when the next one is born." Similarly, with regard to adults the history we have obtained in many of our cases of very fatty liver is that either the male has been unemployed for several months after an uninterrupted period of steady employment during which he was able to purchase reasonably good food, or the female has moved into the "locations" or native slums attached to large towns, where it is difficult to obtain those natural foods which are available to many living on the land.

The type 1a liver, with the fat diffusely scattered as multiple coarse droplets in the cells throughout the entire lobule (fig. 1), is a liver in the early stages of an acute attack. This we were able to corroborate in our previous studies in which the administration of vitamins in conjunction with a full diet intensified the lesions with the result that the multiple fat droplets fused to form a single large globule filling the entire cell. Such a liver is depicted in figure 2 and represents the variety of type 1b. This variety is usually encountered in infants and children dying from malnutrition. It is this liver which when encountered in young pellagrins irrespective of the nature of the clinical condition spells a most gloomy prognosis unless the patient is treated with desiccated stomach.[4]

The next four subtypes (c to f, figs. 3 to 6) represent stages in the absorption of fat. Such livers may not necessarily pass through the type 1b stage, but may be short circuited from the type 1a, depending on the degree of severity of the dietary imbalance or the availability of correct food during the early stages of the disease.

The resolution of the disease may be accomplished slowly, in which case the fat is first gradually absorbed from the central zone of the lobule, where the fat becomes finely dispersed in the form of round, rather dense droplets resembling colloid. In the midzone the fat remains for a much longer time in the form of coarse droplets, while around the portal tracts the globules remain large and are usually enclosed in vacuoles. This is the liver described in foregoing pages as type 1c (fig. 3). In a later stage of healing, the fat gradually disappears, with the result that the large periportal globules remain to outline precisely the portal tracts (type 1d) and, in addition, lipoid globules are

11. Williams, C. D.: Lancet **2**:1151, 1935.

still present in the midzone of the lobule (fig. 4). Those livers in which only the large globules remain, even when these become reduced in size, represent originally extensively fatty livers in which resolution has been imperfect and very slow. Frequently adults with such livers are admitted with clinically rather severe cutaneous lesions, while children whose livers are of the same type are discharged as clinically cured. In both instances, however, there is little doubt that the liver is damaged and cannot be regarded as being normal. The continental pathologists [12] have described such livers as occurring in healthy people dying unnaturally. In numerous biopsies of the livers of "normal" persons we have not thus far seen large droplets of fat in the liver cells. The Kupffer cells, however, normally always contain fine droplets of fat, and in an occasional liver cell the cytoplasm may be packed with small globules of fat. Even these occasional cells must be regarded as abnormal and perhaps represent dying cells, which are known to occur in all glandular organs functioning actively. Even a single large globule of the variety depicted in figure 13, when seen in a section, in our opinion represents some form of hepatic damage even though the injury may be mild. This we have reason to state from work on baboons repeatedly poisoned with small doses of carbon tetrachloride.

The type 1e liver, with its small accumulation of colloid-like fat in the periportal liver cells (fig. 5), shows almost the terminal phase in the slow healing of the liver and is not uncommonly found in children when they leave the hospital after vigorous treatment. But this type, as well as type 1f (fig. 6), may be depleted of all visible fat when the patient is treated effectively, and for this reason both have been regarded as pathologic livers.

In our series, when infants and even adults with a type 1b liver were seen during the acute stages of the disease, the administration of powdered stomach together with a full diet definitely prevented the persistence of the large fat globules in the cells near the portal tracts. Within a few days of such treatment the fat is sharply reduced, and it then becomes distributed chiefly in the form of fine particles with transition to rather coarse globules exceeding the size of the nucleus. After a further ten days, even though the powdered stomach is not administered for more than five days, the fat becomes still further reduced and only an occasional cell contains fine droplets

of deeply stained fat. The cytoplasm of the liver cells is watery.

This rapid disappearance of fat from the liver during the therapy and the accumulation of rather densely staining fat with liver extract and, more especially, with other forms of therapy allow us to conclude that the persistence of the fat in large globules, particularly around the portal tract and even elsewhere in the lobule, is an indication not only that the disease is not resolving naturally but that it is passing gradually into a more chronic form. It is not inconceivable that patients with a type 1a or a type 1b lesion in the liver may have access to food which, while not properly balanced, is able to prevent further progress of the disease and even lead to slow partial healing. The disease in these patients probably heals so slowly that the persistent symptoms of malnutrition eventually bring the patient to the hospital. Biopsy of the liver still reveals considerable damage, which may now be classified as types 1c to 1f.

The persistence of fat in the liver cannot be regarded as being inconsequential, although we have not as yet had the opportunity of following up every patient discharged from the hospital with an incompletely resolved fatty liver. However, it has been repeatedly shown in animals that a long-standing fatty liver may ultimately become cirrhotic.[3] In our experiments in rats we have demonstrated that the fatty transformation invariably precedes by months the cirrhotic changes which result from a diet of mealie pap and sour milk.[5] We have already encountered in a child of 8 years well marked portal cirrhosis resembling that produced in our rats by dietary methods. It is more than likely from the reports published to date that infantile pellagra, so common in India and described in other parts of the world,[13] is preceded by a fatty change in the liver which later may pass into cirrhosis leading eventually to premature death.

As already indicated in the descriptive section of this paper, the reaction of the liver in pellagra is significantly modified by age. This is also reflected in the clinical picture.[14] The livers of pellagrous infants are invariably fatty to a greater or lesser extent. We have examined thus far 27 children below the age of 6 years and have not seen in the liver of a single one any disturbance in the metabolism of iron. At no stage of the healing process have we observed the appearance of intracellular iron pigment or hemofuscin, so common in the adult. Apparently, the fatty liver of the infantile pellagrin, in

12. Rössle, R., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, ed. 2, Berlin, Julius Springer, 1924, p. 278.

13. Rao, P. K.: Proc. Indian Acad. Sc. **14**:310, 1941.

14. Kark, S. L.: South African J. M. Sc. **8**:106, 1943. Gillman, J., and Gillman, T.: To be published.

South Africa at least, does not form any iron even when it is extremely fatty or when the lesion is moderately chronic. Not until after the age of 8 to 10 years does iron appear in sufficient concentration to be identified with our modified technic. After this age, however, the clinical manifestations also assume their adult pattern and for the first time the Casal necklace shows itself, together with the symmetric varieties of cutaneous lesions, as well as the raw, red, beefy tongue and other signs so typical of the adult form of the disease.

Hemosiderin has been observed in the livers of infants and children, especially in association with fibrocystic disease of the pancreas.[15] As far as adult pellagrins are concerned, we may state as a general rule that pigment invariably appears at one stage or another during the course of the disease irrespective of the other features of the pathologic process. The pigment is formed in the cell at a time which coincides with the disappearance of the fat. The degree of pigmentation may vary from case to case. The extent and the intensity of the pigmentation of the liver constitute an excellent indicator of the degree of chronicity of the disease. In no case up to the present have we failed to elicit evidence of a previous attack of pellagra in an adult whose liver shows the presence of considerable amounts of pigment. By this we do not imply that pigment is found only in pellagrins. It is conceivable that other diseases may excite this reaction, but we are convinced from our experience that one of the most fruitful causes of pigmentation of the livers of Africans is pellagra and other varieties of acute and chronic malnutrition.

The iron may accumulate in the liver cell until approximately the whole area distal to the nucleus is filled with rather coarse iron particles. However, even at this stage the iron pigment is by no means permanent, as under treatment it may be gradually reduced until only a few granules remain lining the biliary pole of the cell. The cytoplasm of such cells may have a blue-green flush in appropriately stained sections.

Owing to the fact that most pellagrous patients are recruited from the lowest economic levels, at which they are unable to feed themselves adequately, the livers of these patients steadily deteriorate. In several such patients who were followed up at intervals of four to fifteen months it is evident that the pigment either persists in the cell in quantity similar to that seen during the first and second attacks or

15. Blackfan, K. D., and Wolbach, S. B.: J. Pediat. 3:679, 1933. Andersen, D. H.: Am. J. Dis. Child. 56:344, 1938.

steadily accumulates to pack the cells completely with associated disorganization of the cytoplasmic organoids.

In the early stages of the disease the pigment is restricted to the hepatic cells and to some of the Kupffer cells lining the sinusoids (figs. 7 and 16). Such livers belong to type 2. With the progress of the disease the amount of residual pigment in the liver cells accumulates progressively, especially in the periportal region of the lobule (figs. 8 and 9). The cells may enlarge enormously, and under low power magnification clumps of pigment-filled cells may be seen scattered through the liver. The Kupffer cells may now become heavily pigmented. The portal tracts, too, soon show the massing of pigment. The liver is then classified as type 3. All transitional stages from type 3 to type 4 or the cirrhotic stage exist (compare figures 8 and 9 with figures 10 and 11). The portal tracts become progressively thickened because of the increase in fibrous tissue and the enormous deposition of pigment (figs. 10 and 11). The lobules are outlined in preparations stained for iron by the pigment lodged in grossly enlarged histiocytes. The pigment is heaviest in the liver cells around the portal tracts, but almost all the cells throughout the lobules contain some pigment. The reticulum of the sinusoids near the portal tracts becomes prominent. The main emphasis of the fibrocellular reaction is in the portal tracts. There the fibroblasts, the lymphocytes and the pigment-containing cells are most numerous.

The hepatic cells at the periphery of the lobules are slowly destroyed, and the accumulated pigment is taken up by histiocytes as well as by the lymphatic vessels. However, proliferation of the hepatic cells does occur apparently, as the lobule may enlarge and bulge in the direction of the much thickened portal tracts. At this stage the rusty brown appearance of the liver and the cirrhosis can be easily recognized macroscopically even in the small fragments of tissue removed with the biopsy punch.

In the development of pigmentary cirrhosis it is exceedingly difficult to state with precision when the liver can be regarded as cirrhotic. In the well advanced stage it is quite easy to recognize the cirrhosis (figs. 10 and 11). The intermediate stages provide the greatest amount of embarrassment. Nevertheless, in adult pellagrins the formation of excessive quantities of iron-containing pigment is a consistent finding. In the 120 patients examined to date every desirable stage in the evolution of pigmentary cirrhosis was available to us. We have a complete series of livers from the mildest type 2, in

which hepatocellular pigment appears in small concentration (fig. 7), to the heavily pigmented and cirrhotic type 4 (fig. 11).

Livers of type 2 to type 4 may undergo at any stage a fatty change such as that described in the type 1 series (figs. 19 and 20). Pigmented livers are invariably encountered in patients suffering from subacute or chronic pellagra, but the fatty changes are usually associated with an acute attack.

Thus, in the evolution of pigmentary cirrhosis, one of the terminal results of pellagra, the liver becomes intensely fatty during an acute attack. The fat is recognized at first as fine globules, which arise in any part of the cell. There is no strict localization of the lipoid droplets (fig. 1). They may then fuse to form larger droplets (type 1a), and these in turn may ultimately give rise to the huge droplet found in almost every liver cell as described in type 1b (fig. 2). Thereafter, depending on the nature of the treatment, which in turn affects the rapidity of healing, either the fat may be rapidly swept out of the liver, only a few droplets remaining, as in type 1e or f livers (figs. 5 and 6) or, in less effectively treated patients, the fat is slowly absorbed, first from the cells situated in the central zone and the midzone of the lobule, with persistence for a variable length of time of the large colloidal fat globules in the periportal zone as in types 1c and d (figs. 3 and 4). Clinically such patients may be greatly improved, and they are discharged as cured. However, in following such livers it is seen that a liver of type 1d may ultimately become one of type 1e or f.

In the majority of adult and adolescent pellagrins, coincident with the disappearance of fat iron pigment is almost invariably formed in the hepatic cell, at first strictly localized to the area immediately distal to the nucleus near the biliary pole (fig. 16). In severe pellagra pigment may be formed throughout the lobule, but in general it arises first in those cells nearest the central vein and then progressively involves the rest of the lobule with increasing intensity until the most heavily pigmented cells are observed in the periportal region (fig. 18). The pigment from the central and middle zones may be absorbed. In a second attack of pellagra, the fat again accumulates in the cells, but this time it is not difficult to identify the iron-containing pigment remaining from a previous attack in many of the liver cells containing fat (figs. 19 and 20). The pigment, together with the nucleus and other organoids, may be pushed against the cell membrane (figs. 20 and 21).

From the study of many of our patients who were carefully followed up for fifteen months we can state with some degree of certainty that pigment is the evidence of a previous attack and fat of a superimposed recurrent acute attack which brings the patient back to hospital. As the fat is absorbed, more iron pigment is deposited, and this time some of the liver cells may contain coarse clumps of iron pigment scattered throughout the cytoplasm. These are the cells which are slowly destroyed. With each successive acute exacerbation or with persistence of chronic malnutrition more iron is formed until ultimately the pigment begins to accumulate in the portal tracts, which have become thickened coincidentally. Even in well advanced cirrhosis associated with extensive pigmentation the liver cells do not apparently lose their capacity to form fat under provocation. But in pellagra, as in other diseases, patients do not always present themselves for treatment during the acute stages. In malnutrition it often happens that the patient may accidentally have access to some varieties of food which may alleviate the acute symptoms. Only after experiencing several subclinical attacks may the patient seek treatment. A glance at the table reveals the structure of the liver of the pellagrin when he arrives at the hospital as observed in 120 cases, and it is evident that in almost 40 per cent the disease was in either the subacute or the chronic stage.

We have been fortunate to watch the development of early pigmentary cirrhosis in a patient who suffered from repeated attacks of pellagra. The liver passed progressively through the various stages expressed by livers of types 1a to f and through the stages observed in livers of type 2 and type 3 until pigmentary cirrhosis (observed in type 4) was diagnosed without any doubt. Similarly patients may be admitted with a liver of type 2 and may ultimately be readmitted several months later with a liver of type 3 or type 4.

The grading of the livers into types and subtypes on the evidence already mentioned is more than fully justified. It represents a dynamic expression of the progress of the lesion and, even more important, it provides standards for grading livers, without which all attempts at evaluating therapy must be extremely misleading, especially as the clinical condition does not parallel the extent of the damage in the liver.

We have demonstrated on a previous occasion that livers of types 1a and 1c (previously named livers of the second and third categories respectively[4]) respond to treatment differently from livers of type 1b (previously called livers of the

first category). The livers of types 1a and 1c may sometimes be depleted of fat by the administration of a full diet alone, while the livers of type 1b appear to be aggravated by vitamins and a full diet but respond partially to liver extract and very dramatically to powdered stomach in conjunction with a full diet.[4]

The many remarkable claims for the healing properties of vitamins in pellagra are in our opinion with few exceptions unwarranted, first, because the disease might be in a stage in which it will respond clinically to a well balanced diet alone, as it did in many of our own cases, and secondly, because no criteria other than clinical ones have been used to gage the severity of the disease on admission to hospital. The clinical findings, as we have demonstrated, particularly in children, may not always be reliable, as children may be admitted to hospital with a grave prognosis and yet biopsy of the liver may indicate that the pathologic condition is by no means as serious as the clinical findings seemed to suggest. Moreover, from the histologic appearance of the liver it was possible to predict and subsequently prove that in many of these cases the patient responds to a full diet alone as well as, if not better than, to such a diet in conjunction with the enormous quantities of vitamins recommended. In the absence of any information concerning the structure of the liver, when the patient shows an improved condition after receiving vitamins and a balanced diet, almost invariably the diet is forgotten and the conclusion is drawn that the vitamins have cured a seriously ill patient.

Up to the present, of course, there has been no satisfactory method for assessing the severity of the disease other than that of clinical observation. The biochemical tests have on occasion proved helpful. Now that a safe biopsy procedure is available there is no reason why therapy should not be controlled even more thoroughly than was possible hitherto. The liver is known to be involved in a large number of nutritional diseases. By first carefully grading the livers of patients in whom various specific forms of therapy are to be assessed, those with a comparable degree of damage can be selected and thereafter the reactivity of the liver, as well as the improvement or the deterioration of the clinical condition, can be compared.

In our large series of cases of pellagra we have found that it is much easier to improve the clinical condition of the patient than it is to restore the liver to normal. At this juncture we wish to make it perfectly clear that we do not regard the hepatic damage as responsible for all the

manifestations of pellagra. The liver participates in the disease process to a considerable degree and is not as easily healed as, for example, the sore tongue, the cheilosis or the rash. However, we have no hesitation in stating that when the liver is severely injured it may intensify the disease or even alter the character of the disease process.

It is widely reported that the liberal use of potent vitamin concentrates has been associated with clinical improvement in many cases, and since the internal organs have not been available for histologic examination, the impression remains that in such cases the patient is cured. It has been suspected that in man the liver might be involved in nutritional disease, but no positive evidence has so far been adduced in support of this suspicion.[3a, b] The evidence from our study leaves no shadow of doubt that the liver can be severely affected by nutritional imbalance. In fact, in only 1 per cent of our pellagrins was the liver free from recognizable injury, whereas in 12.5 per cent it was cirrhotic, while in 20 per cent it was precirrhotic. We have also been able to demonstrate that even during treatment, while the patient shows symptomatic and clinical improvement, the pigment continues to be deposited within the cells. We are satisfied, too, that while good food alone or in combination with vitamins can alleviate an acute exacerbation as judged clinically, it has not been able thus far to heal a cirrhotic liver or (except in children) to prevent the deposition of pigment.

The ravages of pellagra appear truly devastating when it is realized that among the 120 persons with pellagra 12.5 per cent suffered from frank pigmentary cirrhosis. If we exclude the children and infants, the incidence of pigmentary cirrhosis in adult Africans suffering from frank malnutrition as determined by the biopsy method is 15 per cent, and we suspect that it may be nearer to 30 per cent.

As far as we have been able to ascertain, there is no known treatment which can repair the damage of the liver caused by pellagra, although of course clinical improvement is the rule. From other evidence, which will be demonstrated at a later stage, we do not feel that it is impossible to cause the removal of fibrous tissue, but as far as the treatment of cirrhosis is concerned we are still unable to restore to normal or even improve a liver showing advanced cirrhosis. At best we may be able to arrest the progress of the disease, but this cannot be achieved by means of large concentrations of vitamins. Actually the deposition of

pigment continues actively in the presence of a mixture of nicotinic acid, riboflavin, thiamine and brewers' yeast. Deposition of excessive pigment in a young person is an indicator of active disease of the liver, and if it is not arrested portal cirrhosis develops simultaneously with the increase in pigment.

This steady deterioration which we have repeatedly observed in the livers of pellagrins may be the explanation of the relapses so common in this disease. It is also an indication that present forms of therapy are unsatisfactory.

PIGMENTARY CIRRHOSIS

The origin of the pigment in hemochromatosis has excited much interest. There is no need to review all the theories propounded to explain the deposition of iron in the cells of the liver and other organs. This has been admirably performed by Sheldon [5] whose handling of this complicated issue must be regarded as one of the most logical analyses presented in recent years. After subjecting the various theories to a searching examination he was led to conclude that a metabolic disturbance in the cell itself was directly responsible for the deposition of iron. This altered metabolism, Sheldon maintained, was not necessarily restricted to the liver cell but probably involved all the cells of the body "with the possible exception of the nervous tissue." Owing to the difficulty of accounting for the peculiar sex incidence of pigmentary cirrhosis, Sheldon was led to conclude tentatively that this disturbed metabolism was inherited and affected chiefly the male sex. He was unable to state with certainty the exact nature of the metabolic error underlying the production of pigments.

According to Sheldon, the age incidence, the sex incidence, the deposits of hemofuscin and hemosiderin and the cirrhotic changes, as well as the accumulation of copper, must all find a place in a satisfactory explanation of the genesis of hemochromatosis.

In discussing hemochromatosis it must be mentioned at the outset that almost all the information thus far available concerning the nature of the tissue reactions has been obtained from postmortem material. Up to the present no person has been able to watch the progress of this disease in the way we were privileged to do as a result of the perfection of the liver biopsy technic. It was possible for us to make a diagnosis from the liver tissue and then proceed to study the patient clinically.

Only one of the triad of hemochromatosis was not recognized, namely, the bronzing of the skin. It must be remembered that the only material available to us was that passing through the Non-European Hospital, which admits only African (natives) and Eur-African (colored) patients. Owing to the natural pigmentation of the skin, it would obviously be exceedingly difficult to recognize any bronzing. Most of these patients, however, did exhibit the typically highly pigmented cutaneous lesions of pellagra. Sheldon himself mentioned that pigmentation of the skin is not an essential feature of the disease; from the statistics compiled he stated that it was absent in 20 per cent of the patients. A profound disturbance in the carbohydrate metabolism was almost invariably established in our patients with moderate pigmentation of the liver. Of course, we could not determine the extent of involvement of other organs in patients subjected to aspiration of liver, but in many of the adult patients with well marked pigmentation of the liver the dorsum of the tongue, too, was found to have one or more patches of black pigment. This pigment we have shown to be iron pigment lodged in the cells of the tunica propria, immediately deep to the epithelium. As pointed out in a previous publication, we regard this pigmentation of the tongue in Africans as being one of the early clinical manifestations of hemochromatosis.[4c] In a series of 200 autopsies conducted on so-called healthy Negroes who had met sudden violent death we have seen livers similar to those encountered in our patients, and there was gross rusty brown pigmentation elsewhere, especially in the upper abdominal group of lymph glands, which gave a strongly positive test for iron. The autopsy observations of this series, together with a report on 500 livers removed in consecutive postmortem examinations conducted on African patients dying suddenly from a variety of diseases, will be more fully reported elsewhere. Sufficient to state at this juncture that we are satisfied that we are dealing with hemochromatosis and that it is a common disease in Africans in Johannesburg.

In the 120 cases of pellagra we saw no evidence of increased destruction of blood since the blood count was usually within the range of normality and the icteric index was normal. The stools and urines were systematically examined for schistosomiasis with negative results. Tapeworms and roundworms (Ascaris) were frequently found, but up to the present neither of these worms has been reported to cause hemochromatosis. Excessive destruction of blood, therefore, could not account for the enormous deposits of intracellular iron seen in the liver. Besides, the Kupffer cells, as a rule, become pigmented only after the liver cells have begun

to accumulate hemosiderin (fig. 22). Some of the Kupffer cells exhibited granules of iron-containing pigment almost at the same time at which iron was identified in the liver cells. Later, as the distended liver cells were gradually destroyed, the iron was taken up largely by the Kupffer cells, which in turn also underwent enlargement. The liver cells as well as the Kupffer cells may deposit iron in the cytoplasm as a result of a common underlying metabolic disease.

The excellence of our material, especially since we were able to examine freshly removed tissue, facilitated the carrying out of several microchemical tests. The mitochondrial preparations in particular were outstandingly beautiful. We are reasonably satisfied that the iron develops on, in or from the mitochondrial elements as previously reported.[16]

The earliest evidence of the appearance of iron is preceded by visible changes in the mitochondria lying immediately distal to the nucleus. In hematoxylin-eosin preparations the particles of iron have a yellowish appearance which may become deeper, even to brown or black. In preparations to demonstrate iron but counterstained with basic fuchsin, pale pink globules indistinguishable from hemofucsin are already present. However, in sudan IV–treated sections, previously stained by our modified technic for iron, we were able to demonstrate that this hemofuscin was indeed of a lipoidal nature. The lipoidal hemofuscin appeared to be colored on the yellow side of red and was a little paler than the other lipoidal droplets present in the cell. This was especially evident in the cells of livers of type 2 or 3 in which fat had accumulated in cells previously containing pigment. By using the combined iron and fat stain we could find no intracellular pigmented elements other than those containing fat or iron. In hematoxylin-eosin preparations we often encountered livers in which the pigment was black, the type of pigment which resembles melanin, but in no instance have we failed to prove the presence of iron even in these black particles. It must be remembered in this connection that Sheldon states that with the improvement of technics for displaying iron, more iron and less hemofuscin were found as compared with the results obtained in the same specimen on a previous occasion. The yellow-brown pigment usually associated with the iron-containing pigment is different from the other fats which are seen in sudan IV preparations insofar as the yellow-

brown granules are not completely dissolved out by alcohols and chloroform during the dehydration and clearing procedures prior to embedding in wax. The yellow-brown pigment appears to be lipoprotein, which stains as well in frozen sections with sudan IV as it does with basic aniline dyes in paraffin sections.

Hemofuscin and hemosiderin appear more or less simultaneously in the cell, but there is some evidence that the hemofuscin may disappear much more easily than the iron. From the evidence available from a variety of technics applied to excellently preserved tissue we are led to conclude that both the hemosiderin and the hemofuscin are derived from preexisting elements (mitochondria) within the liver cell. For this reason we feel that the present nomenclature is unsatisfactory, as it focuses attention erroneously on a blood origin of these pigments. As a radical departure, making the break with the old concepts complete, we would suggest that hemofuscin be known as cytolipochrome and hemosiderin as cytosiderin.

As to the precise origin of the cytosiderin and the cytolipochrome, we have already indicated that both these pigments make their appearance in intimate relation with the mitochondria. Moreover, as the pigments increase there is a corresponding reduction in the mitochondria until the stage is reached when the cell is crammed full of pigment only. At this time it is impossible to identify any mitochondria in the cell, and such cells lose their capacity to form fat. Arnold[17] regarded the iron as a derivative of the plasmasomes (mitochondria), while Gilbert and Surmont[18] stated that the iron is in organic combination with the mitochondria. In our own studies we have been able to show that mitochondria definitely contain iron, and more recently Claude[19] claimed to have demonstrated that mitochondria contain cytochrome-oxidase, as well as copper. Mitochondria are known to contain lipoproteins,[20] and from our cytologic and chemical studies, in conjunction with Claude's findings,[19] we are led to conclude that both the cytosiderin and the cytolipochrome, as well as copper, have a common origin from mitochondrial elements. If Claude's work should

16. Gillman, T., and Gillman, J.: Nature, London **154**:148, 1944.

17. Arnold, J.: Virchows Arch. f. path. Anat. **161**: 384, 1900.

18. Gilbert, A., and Surmont, H.: Paris méd. **39**: 161, 1921.

19. Claude, A., in (a) Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1941, vol. 9, p. 263; (b) J. Exper. Med. **80**:19, 1944.

20. Bourne, G.: Cytology and Cell Physiology, London, Oxford University Press, 1942.

be confirmed, that the mitochondria contain cytochrome-oxidase, ribonuclein and succinic dehydrogenase, as well as phospholipids and copper, then a new important link would be established between biochemistry and morphology. The mitochondria in the liver undergo distinct changes in pellagra, and it may soon be possible to employ morphologic indicators in tracking down some of the obscure biochemical phenomena which are known to occur in normal and diseased cells.

The topographic relation of the cytosiderin and the cytolipochrome suggests that either the iron in conjunction with a protein forms the outer film of the mitochondria, enclosing a core containing the lipochrome in combination with the protein, or the iron-containing fraction is situated within the mitochondrium but alters its position as soon as the disintegration of the mitochondrium commences. Whichever position it occupies, it is evident that once the complicated structure is disrupted, the iron-containing fraction at first forms a coat to the lipoidal fraction. For a while at least this intimate relationship is maintained, and then the cytolipochrome may be recognized apart from the iron pigment. Such round globules then stain a reddish brown or yellow color and may be absorbed, or they may fuse with the neighboring particles to form an irregular mass of yellow pigment such as is often seen in the epithelium of the prostate, the seminal vesicle or the vas deferens. It never assumes as deep a color as does the iron pigment. However, it is apparent from histochemical procedures that both the iron pigment in the early stages of its formation and the cytolipochrome are bound with a varying amount of protein.

In putrefaction in pigmented human livers the iron granules undergo autolysis and the iron itself diffuses throughout the cell, which then stains a diffuse blue-green color with the alcoholic acid-ferrocyanide solution. This seems to add further evidence in support of the view that even when the iron can be demonstrated it is still bound with some other organic substances.[21] By our technics we have demonstrated that intracellular iron can exist in distinct forms, namely: in a masked form in which it cannot be demonstrated microscopically by known technics but is known to be present chemically; second, in combination with organic material and demonstrable by ordinary methods; third, in such a chemical combination that it can be identified only microscopically after hydrolysis with hot acids, and, finally, in the form which is produced when tissues containing the second and third varieties are allowed to undergo putrefaction.

The fact that there is a gross disturbance of the metabolism of intracellular iron in pellagra may also provide an important clue in solving the problem of the porphyrinuria which has been described in this disease. Work is already under way to determine the site of origin of the porphyrin.

Thus the most serious impasse in understanding hemochromatosis is now bridged since the simultaneous increase in cytosiderin, cytolipochrome and copper can be explained on the basis of a profound disturbance of intracellular metabolism having as one of its effects the disruption of the mitochondria. This in turn is responsible simultaneously for the accumulation of iron pigment (cytosiderin), cytolipochrome and copper, all of which are said to be constituents of the mitochondria. This then satisfies one of Sheldon's main desiderata for a sound explanation of hemochromatosis.

As to the age incidence, up to the present we have not seen the formation of iron in children under the age of 8 years. Irrespective of the degree of severity of the fatty change in the liver, iron pigment was not encountered in the liver in a single one of the 27 children on whom repeated biopsies were performed. In this connection it is worthy of comment that a pigmented tongue such as occurs in many adult pellagrins has not been seen by us in children. Apparently there is some evidence to indicate that the retention of iron can be affected by age. Zondek and Karp [22] recorded low values for iron in young animals and a great increase of this element in older ones. However, Ramage, Sheldon and Sheldon [23] found that the iron content of the liver of the infant fell during the nursing period and that thereafter there was a slight rise until the twelfth year. Zondek and Karp suggested that the iron which accumulated in the midperiod of life was in a form other than that previously present in the cell.

While the amount of tissue obtained by the aspiration technic is insufficient to allow quantitative estimation of iron to be made with the methods available to us, it is obvious that the livers of infants and children react differently from those of adolescents and adults in respect to the iron metabolism. Iron appears first at the age of 8 years and then only in small quantities. But thereafter in pellagra the pigment increases rapidly in amount, and in some cases, between the second and third decades, a well marked pigmentary cirrhosis can become mani-

21. Cook, S. F.: J. Biol. Chem. **97**:215, 1932.

22. Zondek, S. G., and Karp, J.: Biochem. J. **28**: 587, 1934.

23. Ramage, H.; Sheldon, J. H., and Sheldon, W.: Proc. Roy. Soc., London, s.B **113**:308, 1933.

fest. The evidence available to date from our pellagrins seems to indicate that there is a distinct difference in the metabolism of intracellular iron in African children and in adults, and further study of this issue may elucidate the reasons why in children with pellagra intracellular pigment does not develop as readily as in adults.

While our experience is similar to that of Sheldon in that we have not encountered pigmentary cirrhosis in children, it is abundantly patent that pigmentary cirrhosis develops in Africans at least ten to fifteen years earlier than in Europeans.

Sheldon has attempted to estimate the time taken for iron to accumulate in the liver on the basis of an average intake of iron. He suggested that it would take at least twenty-eight years for iron to accumulate in the liver commensurate with that usually found in hemochromatosis. Such an argument can be extremely misleading, as it does not take into account the altered metabolism which expresses itself as hemochromatosis. The daily retention of iron must be much greater than the amount Sheldon has arbitrarily selected on the basis of his comparison with the needs of healthy persons. All the African patients in whom pigmentary cirrhosis was diagnosed gave a history of having had several previous attacks of pellagra. It is known that metabolism is greatly disturbed in malnutrition, and it is not inconceivable, in view of the evidence of a grossly disturbed metabolism of intracellular iron, that even with a normal intake of iron the retention may be much greater than in normal people and sufficient to account for the early deposition of the excessive amounts of pigment encountered in our cases.

That in many Africans the liver is injured at an early period in life there can be scarcely any doubt. This is clearly evident from what has already been said concerning the nature of the reactions seen in infant pellagrins. That many children leave the hospital as clinically cured but with a damaged liver cannot be denied, as shown by the examination of fragments removed from the livers of these children on the day of discharge from the hospital. The low economic status of the African militates against any possibility of correct feeding. The consistent repeated attacks of frank or subclinical pellagra, each leaving its imprint on the liver, together with the consumption of considerable amounts of iron in mealie pap may create the ideal conditions for the development of pigmentary cirrhosis at least ten to fifteen years earlier than the time so commonly described for Europeans. On the foregoing basis, therefore, it is not necessary to postulate an inborn error of metabolism to account for the high incidence of hemochromatosis. The incidence of pigmentary cirrhosis among the Africans at the General Hospital is much too frequent to make such a theory acceptable.

From our biopsy material we can state emphatically that iron pigment and cirrhosis are encountered as frequently in females as in males. Moreover, since we have been able to study all stages in the development of pigmentary cirrhosis, we, too, like Strachan,[9] have encountered patients with intensely pigmented livers without cirrhosis. Consequently we cannot regard Sheldon's second criticism of Strachan's findings as valid since, as we have clearly indicated, it is possible at certain stages of the disease to distinguish between extensive deposition of pigment and pigmentary cirrhosis. In adults, at least, the deposition of pigment may precede the cirrhotic process by months if not years. However, Strachan's thesis was not available to us, and consequently we are not in a position to question Sheldon's other criticisms of this work. Since most of the cases which Sheldon reviewed were first diagnosed at necropsy, as were Strachan's cases, it is probable that the lesions were first seen when the deposition of pigment or the cirrhosis or both were already advanced. In such instances any attempt to explain the genesis of the disease must be open to serious criticism.

While our biopsy material demonstrates the high incidence of pigmentary cirrhosis in males and females, we wish to emphasize that we have never seen a female patient manifesting all the signs of advanced atrophic cirrhosis, such as distended abdominal veins and ascites, so frequently observed in the male. It would appear, therefore, that although pigmentary cirrhosis is equally common in males and females suffering from pellagra, nevertheless the lesion does not progress as rapidly in females as in males.

That there is a sex difference in the reactivity of the liver has been repeatedly emphasized in experimental work. Best and Ridout [24] remarked that only female rats are suitable for the production of certain types of fatty livers. In our experiments with mealie pap and sour milk we found that fatty livers and cirrhosis occurred far more frequently among males than females. Moreover, a dose of chloroform which consistently produces fatty changes in the livers of normal female rats fails to do so after castration.

24. Best, C. H., and Ridout, J. H.: J. Physiol. **94**: 47, 1938.

Likewise, male baboons are far more sensitive to chloroform before than after castration.[25]

We agree with Sheldon that atrophic pigmentary cirrhosis is more frequently the cause of death in males than in females, and that in fact this sexual difference in the reactivity of the liver is observed under experimental conditions. On the other hand, on the basis of our experience we do not consider that the equally high incidence of pigmentary cirrhosis in males and females is a valid reason for regarding the disease as seen in this country to be different from hemochromatosis. Hemochromatosis has been repeatedly described in females in other countries, and this disease therefore occurs in both sexes albeit that the terminal phases are commoner in males.[5]

ETIOLOGIC CONSIDERATION OF CYTOSIDEROSIS

From the point of view of etiology cytosiderosis has proved insoluble, and despite the profound disturbance of iron metabolism no significant advances have been made other than the recognition that it is not the result of excessive destruction of blood. After his exhaustive review of the literature and in the light of his own experience Sheldon put forward the hypothesis that this disease is the result of an inborn error of metabolism. In our opinion this is the weakest and most unsatisfactory aspect of an otherwise magnificent study.

We have already brought forward sufficient evidence to prove that cytosiderin and cytolipochrome arise in the cell in intimate relationship with mitochondria. In this respect, therefore, cytosiderosis is to be regarded as a profound disturbance of the intracellular metabolism. That this disease is in no way determined by a hereditary factor is apparent from facts already mentioned, namely, that it is a concomitant at least of some forms of malnutrition in South Africa. The degree of pigmentation is indeed a good indicator of the chronicity of the malnutrition. The pigmented cells of the liver can be regarded to be as much a feature of malnutrition as are the cutaneous rash, the cheilosis and the glossitis. The pigmentary reaction in a case of moderately chronic pellagra can be obscured by the fatty change which frequently occurs in acute exacerbations of the disease. Sydenstricker and co-workers,[16] Denton[26] and others[27] have drawn attention to the fatty liver in patients dying from this disease, but Bean, Spies and Blankenhorn,[28] in quoting Gore, reported the association of hemochromatosis in a case of frank pellagra. This association was regarded as incidental, and no attempt has been made thus far to suggest a causal relation between pellagra and hemochromatosis, although pigmentation of the nervous system has been frequently recorded.[29]

Sheldon, from casuistic evidence based on 15 cases, draws the sweeping conclusion that the disease runs in families and is therefore a congenital affliction which manifests itself late in life although it may occur within the first and second decades. It is true that hemochromatosis runs in families; we have seen it in twin sisters and on several occasions in members of the same family and in families living in the same street. It must not be forgotten that malnutrition is widespread among African (native) and Eur-African (colored) people in South Africa and that under these conditions the incidence of pellagra is naturally high. It is to be expected, therefore, that members of the same family should suffer from the ravages of a form of starvation and consequently manifest the reaction of pellagra, including pigmentation of the liver cells. In infants and children under 8 years of age the liver rarely becomes pigmented after the fat has become absorbed. But then in children there are other manifestations of the disease which are different from those in adults especially the frequency of the generalized edema and the high incidence of steatorrhea. As soon as the adult pattern of the disease begins to express itself in adolescents or even in children, simultaneously the metabolism of the cells becomes profoundly altered in such a way that a deposition of cytosiderin and cytolipochrome in the liver can invariably be predicted.

For these and other reasons mentioned we do not regard cytosiderin as an inborn error of metabolism and we feel reasonably certain that tis occurrence is not determined by a genetic factor. On the contrary, cytosiderosis must now be included among the many manifestations of severe and chronic malnutrition. It is frequently associated with many of the clinical features which have now come to be regarded as pellagra.

The diet of the African people consists chiefly of maize, and it has long been suspected that maize contains some toxin which is responsible for the precipitation of pellagra, so common among maize-eating people. Recent work in the United States has indicated that in some areas selenium is found in concentrations high enough to be regarded as adequate to produce toxic

25. Gillman, J., and Gillman, T.: Unpublished data.
26. Denton, J.: Am. J. Trop. Med. 5:173, 1925.
27. Trowell.[2] György.[3a]

28. Bean, W.; Spies, T. D., and Blankenhorn, M. A.: Medicine 23:1, 1944.
29. Langworthy, O. R.: Brain 54:225, 1931.

effects on the organism.[30] Thus far we have not been able to isolate selenium from the urine of pellagrins. If the concentration of selenium is adequate to produce toxic effects, probably it does so against a particular nutritional background as described for experimental animals by Lillie and Smith.[31]

The role of alcohol in the production of the pellagrous syndrome . cannot be overlooked. From adults especially, a history of alcoholism is frequently elicited. In some of our cases in which an intensely fatty liver was found it was at first difficult to decide whether alcohol could not have played an important part in the disease process. There seems little doubt that in many of our cases alcohol played a definite part in precipitating an acute attack of pellagra, but for several reasons it could not be regarded as the only cause of the fatty liver. First, a fatty liver is the rule in pellagrous infants and children, and it is extremely unlikely that in these the morbid changes could be attributed to alcohol. Second, from the adolescent boys and girls no history of alcoholism was obtained after careful questioning, and the parents were also emphatic on this point. Third, pellagra has a remarkable seasonal incidence, described by many overseas investigators,[32] and, finally, despite the numerous cases in which chronic alcoholism has been reported in association with a fatty liver or cirrhosis it is unlikely that the heavy pigmentation found in cytosiderosis would have escaped the pathologists all over the world at a time when so much interest is being focused on hepatic disease. If alcohol is a factor in pigmentary cirrhosis, then it, too, like selenium, can apparently exert this peculiar effect only against a particular nutritional background.

THE RELATION OF FAT AND PIGMENT TO
CIRRHOSIS AND CARCINOMA OF
THE LIVER

The relationship between the deposition of pigment and cirrhosis is of course an important issue. Mild accumulation of pigment is not often associated with any obvious increase in the cirrhotic tissue in the portal tracts, although when it is associated with accumulation of fat in the liver cells there is usually considerable accumulation of round cells as described previously in children.[4a, b] These lymphocytes appear to come and go in a rhythmic fashion, and the number of cells present at any one time is related in some way to the extent of the fatty change. Only in the later stages, when the pigment is in the form of clumps both in many liver cells and in the histiocytes, is there definite evidence of thickening of the portal tract. This may appear to be greater than it really is, owing to the masses of lymphocytes usually found in association with the pigment clumps. Only after the smaller portal tracts become thickened does the reticulum of the sinusoids come into prominence. This is restricted to the sinusoids related to the periportal zone. It is difficult to express any definite opinion at present as to whether or not the pigment is directly responsible for the connective tissue reaction in the portal tracts. It can be said that the fibrosis rarely occurs in these cases in the absence of massive pigmentation, but we have seen cases of cirrhosis in which scarcely any pigment has been present. This applies particularly to cirrhosis in infants. The pigment cannot of itself be considered as the main factor responsible for cirrhosis. It is important to mention that in pellagra we have never encountered any indication that hemorrhagic necrosis was at any stage the precursor of the cirrhosis as seems to be the case in some forms of dietary experiments in animals [3b] and as is suspected to be sometimes the case in man.[3a]

It has been shown repeatedly that in animals a fatty condition of the liver of long standing may lead to the development of cirrhosis.[34] One of the outstanding features of the liver in pellagra, especially in the acute stage, is the considerable amounts of fat. This is especially obvious in children, but even in adults it is a constant feature. In patients pronounced clinically cured, as well as in untreated patients, we have found persistence of considerable amounts of fat in the periportal zones.

The persistence of fat in such livers for a long time and the development of additional fat, due to the inadequate diets on which the native African and Eur-African people are constrained to live, may in turn lead to conditions in which an overgrowth of fibrous tissue becomes possible. Even though the cirrhosis may in some way be related to the fat, it must be mentioned that in pellagra it does not develop in the manner described by Connor in his report on the fatty livers of persons with chronic alcoholism. The cirrhosis in pellagra is restricted to the portal

30. Moxon, A. L., and others: Cereal Chem. **20**: 376, 1943. Moxon, A. L., and Rhian, M.: Physiol. Rev. **23**:305, 1943.

31. Lillie, R. D., and Smith, M. I.: Am. J. Path. **16**:223, 1940.

32. Sydenstricker, V. P., and Thomas, J.: South. M. J. **30**:14, 1937.

33. Himsworth: Personal communication to the authors.

34. György.[3a] Himsworth.[3b] György and Goldblatt.[3c] Gillman and others.[8]

tracts, and vascularization of the sinusoids has not been seen to occur in our cases thus far.

There is every reason to suspect that there may be at least two ways in which portal cirrhosis develops, namely, that described by Connor and observed by us in rats fed mealie pap and sour milk, in which the circumstances are such that some sinusoids dilate and at the same time the walls become thickened by a gradual ingrowth of fibrovascular tissue from the portal tracts, and that in which there is progressive thickening of the portal tracts without any marked disturbance of the sinusoidal pattern even in the early stages. In those livers in which the fibrovascular tissue grows into the lobules there is gross distortion of the structure of the liver and large islands of hepatic tissue may be surrounded by thick bands of fibrous tissue. In the cytosiderotic liver the structure is preserved for a long period. The lobules become grossly accentuated by the thickened portal tracts as in the liver of the pig.

Pigmentary cirrhosis can develop rapidly. In children cirrhosis may develop within three to five years. Not only does the liver become cirrhotic more rapidly in malnutrition but the cirrhosis is encountered at a much earlier age. In Europeans the published statistics indicate that cirrhosis reaches its maximum incidence in persons between the ages of 40 and 55,[12] whereas the overwhelming majority of our patients had cirrhosis fifteen to twenty years earlier. It is not an uncommon event to find a cirrhotic liver in an African male between the ages of 17 and 20 years, nor is the event entirely unexpected. After all, the disease starts early in infancy. Many of those who survive the first attacks are destined, by economic circumstances, to live on a poor diet and under bad hygienic conditions for the rest of their lives. Unfortunately, the liver does not escape injury, and the repeated and continuous insults, inevitable in these circumstances, lead to the development of frank pigmentary cirrhosis in 15 to 30 per cent of our cases.

That the liver of the African is prematurely damaged is indicated by the high incidence of hepatocellular carcinoma in young people. In a series of 292 Africans with carcinoma of an organ other than the liver Berman[35] revealed the startling fact that the carcinoma originated in the liver in no less than 90.5 per cent.

In the last two years of this study no less than 16 cases of primary carcinoma were diagnosed by aspiration biopsy of the liver. One of the patients was only 14 years old, while 4 others were under the age of 20 years.

Cirrhosis and carcinoma in the African are frequently associated with the presence of excess iron pigment. We have already shown that cirrhosis is probably not caused by the iron pigment, and similarly we are of the opinion that carcinoma is not caused by cirrhosis or by the cytosiderin.

However, not every liver, even when intensely fatty, becomes cirrhotic, nor is the cirrhosis associated with a fatty liver necessarily the result of the same stimulus that causes the fatty change. Similarly, not every liver which contains iron pigment necessarily becomes cirrhotic. On the other hand, cirrhosis may occur in any one of the liver types described in the foregoing pages. The conditions necessary for the development of a cytosiderotic liver, as in the instance of the fatty liver, are unknown. The problem of cirrhosis is not the problem of cytosiderosis or of the fatty change but rather of the altered reactions of the interstitial tissue of the liver. A cytosiderotic liver, like a fatty liver, is apparently more liable to become cirrhotic than a liver without fat or iron, but this by no means implies that the accumulation of fat or iron is the cause of the cirrhosis. Cytosiderosis should be regarded as a distinct pathologic reaction not directly related to cirrhosis.

The evidence presented in this study demonstrates conclusively that lack of good food can result in severe disease of the liver in man. The high incidence of hepatic disease in backward and impoverished people recorded by Vint in East Africa[36] and by Bonne and associates[37] in the Netherlands East Indies can therefore now be attributed in large measure to malnutrition. It is apparent that the fatty liver, cytosiderosis with and without cirrhosis, and carcinoma are not consecutive events in the progression of hepatic disease but represent different patterns of reactivity of the liver in response to badly balanced diets.

The excessive deposition of iron in the livers of African patients is of the utmost importance, especially as we have shown that it results from a profound metabolic disturbance of the cell itself, induced by malnutrition. An increase in demonstrable iron and other brown pigments is said to occur naturally in old age.[38] It has also

35. Berman, C.: South African J. M. Sc. **5**:54 and 92, 1940; **6**:11 and 145, 1941.

36. Vint, F. W.: Kenya & East African M. J. **7**:349, 1931.

37. Bonne, C., and others: Proc. Roy. Soc., London, s. B **122**:429, 1931.

38. Pfuhl, W.: The Liver, in von Möllendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1932, vol. 2, p. 235.

been described in animals during hibernation and starvation.[39] When seen in the tissues of young people it cannot be regarded lightly as wear and tear pigment but is an indication of severe damage of the cells. That it is abundant in young Africans there can be little doubt now. If the accumulation of pigment is natural to old age, by the same token the liver cells of Africans must be regarded as manifesting evidence of premature senescence. This premature senescence would account to some extent for the incidence of cirrhosis of the liver at an age at least fifteen to twenty years earlier than that described in other parts of the world where living conditions are much better. It appears that where the incidence of hepatic disease is high there is a corresponding increase in the incidence of carcinoma.[40] The high incidence of carcinoma of the liver in Africans is therefore an expression of the widespread occurrence of hepatic disease.

The lack of adequate food during childhood and adolescence is in no small measure responsible for the early appearance of hepatic disease in Africans. This receives support from our previous work, in which it was shown that the livers of animals fed a balanced diet from weaning until the animals attained 100 Gm. in weight were more slowly and less severely damaged than those of animals fed a bad diet from the day of weaning.[8] It is likely, too, that an unfavorable nutritional background can be created during intrauterine life and during the period of breast feeding.[41]

From this study it is evident that at the present stage of our knowledge the high incidence of acute and chronic damage of the liver, including cirrhosis and carcinoma, cannot be related to any racial factor.

It so happens that at this moment in time the pigmented people are economically backward and, as a consequence, are unable to provide themselves with sufficient amounts of protective foods.

The elaboration of a safe liver biopsy technic allows us to state with a great measure of confidence that the fatty liver and cytosiderosis, with and without cirrhosis, are indeed expressions of severe malnutrition. The high incidence of chronic disease of the liver in young people in South Africa, as well as in other parts of Africa and Asia, reveals some of the devastating effects of malnutrition and calls for drastic

action if life-long ill health and disease, the lot now of millions of people, are to be prevented in the future.

SUMMARY

By an improved liver biopsy technic, fragments of liver have been removed from 120 pellagrins on admission to hospital. It has been established that malnutrition in man causes severe damage of the liver, including pigmentary cirrhosis.

On the basis of the amount, the character and the distribution of fat and pigment and the presence or the absence of cirrhosis these livers have been graded into four main types.

In type 1 the characteristic feature is the presence of varying amounts of fat only. This type may be subdivided into six subtypes according to the quantity, the nature and the distribution of the fat.

Type 2 contains cytosiderin and cytolipochrome in the hepatic and Kupffer cells only, distributed in the form of discrete granules.

Type 3 is very similar to type 2 except that the iron-containing pigment not only is present in the liver cells and the Kupffer cells, as in the type 2 livers, but is aggregated in large masses in cells lying either in the hepatic lobule or in the portal tracts.

Pigmentary cirrhosis of varying intensity is the main feature of type 4.

Livers of types 2, 3 and 4 could exhibit the same degrees of fatty change as described in livers of type 1.

The type 1 liver is predominantly a feature of pellagrous infants and children while the pigmented liver, with or without fat, is found in adolescents and adults.

Frank pigmentary cirrhosis was observed in 12.5 per cent of all pellagrins or 15 per cent of the adults, and there was strong presumptive evidence that the incidence of cirrhosis in this series of pellagrins might be as high as 30 per cent. Pigmentary cirrhosis occurred chiefly in patients under 40 years of age and was indistinguishable clinically and pathologically from hemochromatosis.

Iron pigment arises within the liver cells as the result of a profound disturbance of intracellular metabolism induced by dietary imbalance. Both hemosiderin and hemofuscin have a common origin from mitochondria, and since they arise within the liver cells the names "cytosiderin" and "cytolipochrome" have been suggested to replace the old and misleading terms.

The evidence presented reveals that pigmentary cirrhosis is not the result of an inborn error of metabolism but is one of the manifestations of chronic malnutrition.

39. Kremer, J.: Ztschr. f. mikr.-anat. Forsch. 44: 234, 1938.

40. Karsner, H. T.: Am. J. Clin. Path. 13:569, 1943.

41. Mason, K. E., and Bryan, W. L.: Biochem. J. 32:1785, 1938. Fehily, L.: J. Trop. Med. 44:21, 1941.

Since the liver aspiration technic shows that every pellagrin exhibits a different degree of hepatic damage, the present routine treatment of this disease is unjustified, especially as in many cases the progress of the hepatic lesion continues silently despite the healing of the overt manifestations. Repeated attacks of pellagra may cause a fatty condition of the liver to progress into frank pigmentary cirrhosis. In view of these findings the prescription of therapy and the assessment of its effectiveness should be based not only on the clinical picture but, more especially, on the structural alterations in the liver in each case of pellagra. Since we have demonstrated that severe malnutrition is consistently associated with severe damage of the liver initiated at an early age, the high incidence of cirrhosis and even of primary carcinoma of the liver in relatively young Africans finds a ready explanation.

It is suggested that in pellagrins the fatty change, the formation of intracellular pigment, with or without cirrhosis, and carcinoma represent different patterns of reactivity of the liver cells to acute and chronic malnutrition.

AGING PROCESSES IN THE ENDOCRINE GLANDS OF THE GUINEA PIG

I. THE INFLUENCE OF AGE, SEX AND PREGNANCY ON THE MITOTIC ACTIVITY AND THE HISTOLOGIC STRUCTURE OF THE THYROID, PARATHYROID AND ADRENAL GLANDS

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The present paper represents the first of a series of investigations which have for their purpose the determination of the influence of age on the normal proliferative activity of certain of the endocrine glands, as well as the influence of aging on the response of these glands to various stimuli.

In previous experiments Loeb and I [1] noted a parallelism in the response of the thyroid and parathyroid glands under various experimental conditions. Administration of an acid extract of the anterior lobe of the hypophysis, implantation of hypophysial tissue and feeding of potassium iodide resulted in increased mitotic activity in both the thyroid gland and the parathyroid glands, while underfeeding and administration of thyroid substance resulted in marked diminution of mitotic activity in these glands. In subsequent experiments I [2] was able to confirm the previous observation that a parallelism exists in the effect of potassium iodide on the thyroid and the parathyroid glands and to show that in all probability this substance also produces a slight increase in the mitotic activity of the adrenal cortex.

In the present investigation the changes in mitotic activity as well as the alterations in histologic structure with increasing age are compared in the thyroid, parathyroid and adrenal glands of guinea pigs of both sexes. From this study it can be determined whether or not the parallelism observed previously obtains also

1. Blumenthal, H. T., and Loeb, L.: Endocrinology 30:502, 1942.

2. Blumenthal, H. T.: Endocrinology 31:226, 1942.

under normal conditions with aging as the primary variant, and the results will furnish control data for measuring the response of these glands to various stimuli at different ages.

MATERIAL AND METHOD

The mitotic activity in the thyroid gland, the parathyroid glands and the cortex of the adrenal gland was determined according to methods previously described. [3] The mitotic counts of the cortex of the adrenal gland were recorded as the average number of mitoses per section, those of the thyroid gland as the number of mitoses per gland (both lobes), and those of the parathyroid gland as the number of mitoses per 10,000 cells. In regard to the latter gland the average number of epithelial cells per unit field was also recorded, since this figure indicates changes in the average cell size.

In general, the guinea pigs were divided according to weight, each 100 Gm. in body weight serving as a division point. In most instances the age range corresponding to a given weight group was definitely known, but in some instances only the approximate age could be ascertained.

RESULTS

Influence of Age, Sex and Pregnancy on Mitotic Activity in the Thyroid, Parathyroid and Adrenal Glands.—(a) The Thyroid Gland: Mitotic counts were carried out on the thyroid glands of 142 female and 118 male guinea pigs of various ages, as shown in table 1. From these results it can be seen that mitotic activity is greatest during embryonal life and gradually diminishes with increasing age until it becomes low in very old guinea pigs. The rate of the diminution in mitotic activity (as indicated by the difference in mitotic counts between successive age groups) is greatest between the embryonal period and the earliest period of extrauterine life. Following this there is a gradual decrease in the rate of diminution in mitotic activity until finally the difference between the oldest age group and the preceding age group is relatively small.

It may be observed from the figures in the columns showing the range of variation in individual mitotic counts that there is considerable overlapping between various age groups. This is due mainly to the fact that it is not always possible to make sharp arbitrary demarcations between various age and weight groups, and thus there is probably some overlapping in age groups as well as in the range of variation in

3. Blumenthal, H. T.: Endocrinology 27:477, 1940.

mitotic counts. However, even if we make allowance for this fact, there still remains some overlapping even between more distantly separated age groups, indicating that there are other factors besides age which influence mitotic activity in the thyroid gland. In females the changes in mitotic activity during various stages in the sexual cycle may represent one of these factors.

It may be noted further that mitotic activity is greater in the thyroid glands of females than in those of males of a corresponding age group in all postem-

and 112 male guinea pigs of various ages (table 2). The results here are strikingly parallel to those observed in the case of the thyroid gland. Mitotic activity is greatest during embryonal life and gradually diminishes with increasing age until in old guinea pigs weighing over 600 Gm. it becomes low. As in the thyroid gland, the rate of the diminution of proliferative activity, indicated in the column representing the difference between successive groups, is greatest between the embryonal period and the earliest period of extrauterine life, and then it gradually decreases until

TABLE 1.—*Mitotic Counts on Thyroid Glands of Normal Male and Female Guinea Pigs*

		Male				Female				
Age	Weight, Gm.	Guinea Pigs	Average Mitotic Count	Difference in Mitotic Activity Between Successive Age Groups	Range of Variation in Mitotic Count	Guinea Pigs	Average Mitotic Count	Difference in Mitotic Activity Between Successive Age Groups	Range of Variation in Mitotic Count	Difference Between Female and Male Count
35-50 day embryos*....	9-60	7	2,571.4	......	1,060-4,420	5	1,494.0	.....	1,000-2,100	—1,077.4—(1.7)‡
4 days to 1 mo.*.......	100-199	23	217.0	2,354.4	80- 960	23	560.4	933.6	120-1,600	343.4 (2.6)
1 to 4 mo.*............	200-299	26	134.0	83.0	40- 420	23	379.6	180.8	100-1,480	245.6 (2.8)
4 to 8 mo.*............	300-399	24	96.0	38.0	40- 180	31	225.2	154.4	0-1,080	129.2 (2.3)
8 to 12 mo.*...........	400-499	16	47.5	48.5	0- 140	21	135.2	90.0	20- 330	87.7 (2.8)
12 to 18 mo.*..........	500-599	13	23.1	24.4	0- 100	19	75.8	59.4	0- 280	52.7 (3.3)
1½ to 3 yr.†..........Over 600		9	20.0	3.1	0- 60	20	57.5	18.3	0- 170	37.5 (1.9)
Total..............		118				142				

* The exact age range represented by this group was known.
† Only the approximate age range could be determined for this group.
‡ The figures in parentheses represent the relative difference between the female and the male count (Female Mitotic Count/Male Mitotic Count).

TABLE 2.—*Mitotic Counts on Parathyroid Glands of Normal Male and Female Guinea Pigs*

		Male						Female					
Age	Wt., Gm.	Guinea Pigs	Average Number of Mitoses per 10,000 Cells	Difference in Mitotic Activity Between Successive Age Groups	Average Number of Cells per Unit Field	Range of Variation in Average Number of Mitoses per 10,000 Cells	Range of Variation in Average Number of Cells per Unit Field	Guinea Pigs	Average Number of Mitoses per 10,000 Cells	Difference in Mitotic Activity Between Successive Age Groups	Average Number of Cells per Unit Field	Range of Variation in Average Number of Mitoses per 10,000 Cells	Range of Variation in Average Number of Cells per Unit Field	Difference Between Female and Male Count
35-50 day embryos	9-60	5	23.7	....	211	5.7-47.6	150-253	4	20.4	....	209.5	10-29.4	190-303	3.3 (1.2)
4 days to 1 mo....	100-199	26	3.0	20.7	209.4	0 - 8.0	160-252	26	4.1	16.3	205.1 (±3.63)*	0.9-16.5	165-280	1.1 (1.4)
1 to 4 mo.........	200-299	26	1.6	1.4	218.5	0 - 3.7	150-325	22	2.8	1.3	163.2 (±5.41)	0.4- 8.8	160-236	1.2 (1.3)
4 to 8 mo.........	300-399	21	0.8	0.8	224.8	0 - 2.0	145-326	25	1.3	1.5	187.1 (±4.56)	0 - 3.3	145-266	0.5 (1.6)
8 to 12 mo........	400-499	18	0.7	0.1	192.5	0 - 4.0	140-270	27	1.2	0.1	198.1 (±4.35)	0 - 8.5	160-280	0.5 (1.7)
12 to 18 mo.......	500-599	12	0.6	0.1	177.2	0 - 2.3	148-205	21	0.7	0.5	215.3 (±6.22)	0 - 3.6	140-302	0.1 (1.2)
1½ to 3 yr........Over 600		4	0.3	0.3	203	0 - 1.0	190-232	19	0.6	0.1	214.3 (±4.60)	0 - 2.6	135-259	0.3 (2.0)
		112						144					

* The figure in parentheses is the standard error.

bryonal periods studied. In the group of embryos the average counts were higher in males than in females, but this may be accidental since the number of embryonic thyroid glands representing each sex was small. While the absolute difference in mitotic counts of the thyroid gland in the postembryonal period between female and male guinea pigs of corresponding age groups diminishes with increasing age, the relative difference in mitotic count between females and males (female mitotic count/male mitotic count) remains approximately constant.

(b) The Parathyroid Glands: Mitotic counts were carried out on the parathyroid glands of 144 female

the difference between the three oldest age groups becomes very small. Furthermore, the overlapping in mitotic counts of various age groups as shown in the columns representing the range of variation is present here also, and it is subject to the same explanation as in the case of the thyroid gland.

The parallelism in mitotic activity between the thyroid gland and the parathyroid glands may be noted further from the fact that mitotic activity is greater in the parathyroid glands of female guinea pigs than in those of males of a corresponding age group. The mitotic activity of the parathyroid differs, however, from that observed in the thyroid gland in that the

mitotic counts of female embryos as well as those of the females of every other age group are higher. Since the sex difference is already present before the onset of sexual maturity, it is apparently independent of the activity of the ovaries. While the absolute difference in mitotic activity between the two sexes diminishes with increasing age, the relative sex difference (female mitotic count/male mitotic count) is here, as in the thyroid gland, remarkably constant. The differences in cell number and cell size seem to be variable, yet there is a strong suggestion that the largest cells are present during the period of greatest sexual activity.

(c) The Adrenal Gland: Mitotic counts were carried out on the adrenal glands of 127 male and 134 female guinea pigs of various ages (table 3). In general the results are similar to those observed in the case of the thyroid and parathyroid glands. The counts are highest in embryos and then gradually diminish with increasing age. As in the case of the other two types of glands, the rate of the diminution in mitotic activity is greatest between the embryonal period and the earliest period of extrauterine life; following this, as a rule, a gradual diminution in the

connective tissue about the vessels is likewise loose. Gradually with increasing age the acini become larger, the colloid harder and the epithelium flatter. The stroma becomes fibrous-hyaline in character and appears to be especially dense around arteries; it is less dense about veins, and least dense about lymphatic vessels. These changes occur in almost imperceptible increments, so that it is often difficult to note the differences between successive age groups. But these changes also show overlapping similar to that observed in the variation in mitotic activity between successive age groups. There was no definite demonstrable difference between the two sexes compared at corresponding ages, although the epithelium tended in general to be somewhat higher in females than in males between the ages of 2 and 8 months.

(b) The Parathyroid Gland: The parathyroid gland of the guinea pig embryo is composed of compactly arranged cells enclosed in a thin fibrillar capsule. There are occasional small capillaries with a few adjacent fibrillae. Following birth, the capsule gradually becomes fibrous-hyaline in character with increasing age and sends trabeculae into the substance of the gland. In the older parathyroid gland the arterioles

TABLE 3.—*Mitotic Counts on Adrenal Glands of Normal Male and Female Guinea Pigs of Various Ages*

Age	Weight, Gm.	Male				Female				Difference Between Female and Male Count
		Guinea Pigs	Average Mitotic Count	Difference in Mitotic Activity Between Successive Age Groups	Range of Variation in Mitotic Count	Guinea Pigs	Average Mitotic Count	Difference in Mitotic Activity Between Successive Age Groups	Range of Variation in Mitotic Count	
35-50 day embryos.....	9-60	5	7.3	...	4.3-10	5	7.8	...	2.4-10	0.5 (1.07)*
4 days to 1 mo.........	100-199	26	3.3	4.0	1.2- 9.6	28	5.9	1.9	1.0-16.6	2.6 (1.79)
1 mo. to 4 mo.........	200-299	26	2.8	1.5	0.8-10.0	20	4.7	1.2	1.1- 7.6	1.9 (1.89)
4 mo. to 8 mo.........	300-399	31	2.3	0.5	0- 7.4	20	2.8	1.5	0-10	0.5 (1.22)
8 mo. to 12 mo.........	400-499	24	1.5	0.8	0- 8.7	17	2.1	0.7	0- 8.4	0.6 (1.40)
12 to 18 mo.............	500-599	6	1.2	0.3	0.2- 1.8	19	2.0	0.1	0- 6.8	0.8 (1.67)
1½ to 3 yr.............Over 600		9	0.9	0.3	0 - 2.5	25	1.1	0.9	0- 3.8	0.2 (1.22)
Total..............		127				134				

* Figure in brackets represents ratio of female to male counts.

difference of mitotic activity between successive age groups takes place until finally the difference between the oldest and the preceding age group is relatively small. There is here an overlapping in the range of variation in mitotic counts between various age groups similar to that observed in the case of the thyroid and parathyroid glands, and these variations are subject to the same interpretation as in the case of those glands. Furthermore, the sex difference noted in the thyroid and parathyroid glands is present also in the adrenal glands; in all groups, including the embryos, mitotic counts are higher in female than in male guinea pigs. Again, while the absolute difference in mitotic activity between the two sexes in corresponding age groups diminishes with increasing age, the relative difference remains approximately constant.

Influence of Age, Sex and Pregnancy on the Histologic Structure of the Thyroid, Parathyroid and Adrenal Glands.—(a) The Thyroid Gland: The histologic changes with age which I have observed in the thyroid gland are essentially the same as those which were reported by Loeb and Simpson.[4] In embryo guinea pigs the acini are small, contain soft colloid and are lined with well developed cuboidal epithelium. The stroma is composed of thin fibrillar septums, and the

4. Loeb, L., and Simpson, R. M.: Science **88**:433, 1938.

become more prominent because they are frequently congested and surrounded by some fibrous-hyaline tissue. The changes in size of the epithelial cells with increasing age are shown in table 2. While there is no regularity as to the change in size with age, the cells attain their largest size between the ages of 8 and 18 months in males and 1 and 12 months in females. There is a decrease in size in older animals, corresponding to the diminution in size of the epithelial cells lining the acini of the thyroid gland in older guinea pigs. This is probably a pressure effect caused by hard colloid. In the parathyroid glands of younger animals the average size of the epithelial cells is greater in females than in males; this condition was reversed in older animals. There is no characteristic parallelism between the size of cell and the number of mitoses.

(c) The Adrenal Cortex: In embryo guinea pigs the capsule surrounding the adrenal gland is very thin and is composed of a loose fibrillar connective tissue which sends occasional fine trabeculae into the cortex. The capsule thickens somewhat with increasing age until in old guinea pigs it assumes a fibrous-hyaline character and the septums which penetrate into the zona glomerulosa become somewhat thicker, although they penetrate for only a short distance. There is never in guinea pigs a distinct invasion of the cortex

by connective tissue such as Loeb [5] observed in various inbred strains of mice.

The zona glomerulosa is relatively thicker in embryos than in younger guinea pigs preceding sexual maturity. In young immature and in young adult guinea pigs it becomes relatively thinner with increasing age until the age of about 8 to 12 months, when there is usually a distinctly thicker zona glomerulosa in females, whereas in males the zona glomerulosa shows no increase in number of cells at this age. This difference remains even in old guinea pigs.

The zona fasciculata of the embryo guinea pig is composed of solid cells throughout, but in the young immature animal this zone can be divided into an outer area, which comprises about one third of the thickness of the zona fasciculata and is composed of cells containing fine globules of lipid material in their cytoplasm, and an inner area, which comprises the remaining two thirds of the thickness of this zone and in which the cytoplasm of the cells is more solid. Gradually in almost imperceptible stages, larger vacuoles appear in the outer third of the zona fasciculata; they are probably formed by coalescence of smaller globules, and they increase in number with increasing age. The number of vacuoles is thus in inverse ratio to the degree of mitotic activity, which diminishes with increasing age. A further relation to mitotic activity is shown in the fact that the vacuoles are usually present in greater number in males than in females of corresponding age, although there are some exceptions to this rule.

The zona reticularis is relatively small in embryos and in young immature guinea pigs. It becomes more prominent with increasing age and is composed of clusters of large cells with solid pink-staining cytoplasm. At about the age of 8 to 12 months the cells begin to ingest a brown pigment, which is probably blood pigment. The number of cells containing this pigment gradually increases with increasing age. In addition there is a constant degeneration of the cells. In very old animals large spaces separate groups of cell strands. These spaces are lined with elongated endothelial cells and contain a finely granular coagulum. There are also widely dilated, congested vessels, which extend from the zona reticularis into the medulla. In the guinea pig there is no X zone bordering on the medulla, which is different in the two sexes and which makes it possible to distinguish between them.

COMMENT

According to Loeb, there are two principal types of structural changes which are characteristic of aging processes, namely (1) a gradual decrease of growth processes in various tissues, which may be accompanied by an increase in cell differentiation, and (2) hyalinization and sclerosis of the stroma. The age changes seen in these experiments follow essentially a similar pattern.

The decrease in growth processes here is indicated by the gradual diminution in mitotic activity which takes place with increasing age in the thyroid, parathyroid and adrenal glands of both sexes. Furthermore, there is a parallelism in the rate of diminution in mitotic activity with increasing age in these three glands. The curve of diminution in mitotic activity is not, however, an even one, at least in the female animals. In the case of the thyroid gland Chouke and I [6] observed periodic variations in mitotic activity caused by the recurrent sexual cycles, and these variations may perhaps obtain also in the parathyroid and adrenal glands. There is an antagonism between the factors of aging, which tend to diminish the rate of mitotic activity, and the effects of the female sex hormones, which produce periodic increases in mitotic activity. Whether or not these periodic rises also diminish with increasing age is a question which we hope to answer in subsequent experiments. It has been stated by Carlson [7] that until it is possible to measure quantitatively the amounts of the various hormones circulating in the blood stream, as well as their changes with aging, little progress will be made in determining the age changes in the endocrine system. While such data would be of value, they would not give an insight into the changes taking place with increasing age in the various end organs, which might modify the response of these organs to hormonal stimuli. The latter changes could, however, be determined by administering definite quantities of hormones to animals of various ages and studying the reactions of the end organs to the hormones. The measuring of the mitotic activity may well provide a quantitative index of such changes, as Loeb and his co-workers [8] have shown in previous investigations. Among these end organs may be included the endocrine organs themselves, and the study of the structural changes and the changes in mitotic activity in these organs at different ages in normal animals, as well as in animals to which hormones have been administered, may contribute much to the analysis of aging processes in these organs.

Hyalinization of the stroma with increasing age has also been observed in these experiments, but with varying degrees of intensity in the three glands studied. It is more marked in the thyroid gland than in the parathyroid and adrenal glands, where it is present to only a slight degree. The condition in the guinea pig resembles in this respect that found in the mouse, but on the whole

5. Loeb, L., in Harvey Lectures, 1940-1941, Baltimore, Williams . & Wilkins Company, 1941, vol. 36, p. 228.

6. Chouke, K. S., and Blumenthal, H. T.: Endocrinology 30:511, 1942.

7. Carlson, A., in Cowdry, E. V.: Problems of Aging, Baltimore, Williams & Wilkins Company, 1939.

8. Loeb, L.: Klin. Wchnschr. 51:2121, 1932. Loeb, L., and Haven, F. L.: Anat. Rec. 42:217, 1929. Friedman, H., and Loeb, L.: ibid. 59:5, 1934. Gray, S. H., and Loeb, L.: Am. J. Path. 4:257, 1928.

the changes in the thyroid gland are not as marked in the guinea pig as in certain inbred strains of mice. An interesting change taking place with advancing age is the dilatation of the blood vessels, which was marked in the parathyroid and adrenal glands and which was present, although to a lesser degree, in the thyroid gland. This effect may be due to an alteration within the walls of the arterioles, diminishing their contractility, but it may be due also in some degree to stromal changes around the blood vessels. The hyalinization of the connective tissue attached to the outer coat of the blood vessels may make them less yielding and thus cause resistance to the contraction of the vessel.

Certain other changes noted in the adrenal cortex might influence the proliferative activity in this gland. I refer particularly to the changes in the number of vacuoles in the cells of the zona fasciculata. Loeb and I [9] have recently observed in underfeeding experiments on guinea pigs that the degree of mitotic activity is in inverse ratio to the number of vacuoles present in the cells of the zona fasciculata. This holds true also in the present investigations. In general the adrenal cortex of the female guinea pig contains fewer vacuoles than that of the male of a similar age, and the number of mitoses is correspondingly greater in the female than in the male. With increasing age there are an increase in the number of vacuoles in the zona fasciculata and a diminution in the number of mitoses. As I have stated previously, it thus appears likely that the presence of certain fatty or lipoid substances in cortical cells inhibits their proliferation, which otherwise would take place under the influence of hormones, and that conversely the discharge of lipoid or fatty materials from the adrenal cortex may lead to multiplication of cells in this gland.

These observations concerning the relation of the proliferative activity of the cortex of the adrenal gland to the lipoid content are in accord with the findings of several other investigators. Dosne and Dalton,[10] as well as Selye,[11] have found that the amount of cortical lipoid, demonstrated by either sudan stain or osmic acid, definitely decreases as the adrenal gland enlarges with increased activity. Recently Sarason [12] observed in human subjects with overwhelming infection and cachexia markedly enlarged adrenal glands depleted of lipoid. The latter investigator

also noted depletion of the adrenal cortical lipoids of rats under various experimental conditions, including exposure to high altitudes; the latter factor has been shown by Evans [13] and Lewis, Thorn, Koepf and Dorrance [14] to activate the adrenal cortex. On the other hand, Flexner and Grollman [15] hold that a depression of adrenal activity is associated with a loss of cortical lipoid; this belief is based on the observation that a depletion of the lipoids of the cells of the zona glomerulosa follows administration of an excess of adrenal cortical extract. More specifically related to the present experiments are the findings reported by Whitehead [16] that the proportion of the guinea pig adrenal cortex occupied by lipoid decreases progressively in both sexes from fourteen to one hundred and sixty-eight days after birth and that after fourteen days the adrenal glands of females contain relatively more cortical lipoid than those of males. He measured the thickness of the cortex occupied by fatty substances but did not determine the total amount of these substances, nor the relative number of cells containing fat globules.

The sex differences in mitotic activity which we have observed in these experiments cannot be fully accounted for on the basis of the greater stimulating effect of female than of male sex hormones since these differences were present in as yet sexually immature animals and perhaps even in the adrenal gland of the embryo. The differences observed in embryos are not conclusive since there were too few experiments in this age group. The results in the case of post-embryonic, sexually immature animals suggest, however, that the difference between the sexes is caused by genetic factors rather than by sex hormones of the animals, although the female sex hormones may contribute to this difference during the period of sexual maturity. Whether or not there are sex differences also in embryonic life must be left to further investigation.

One sees then that, beginning with the embryo, through extrauterine life and through adult life to old age a continuous series of changes take place in various organs with internal secretions. These changes consist in (1) a step by step diminution in proliferative activity and (2) alterations in the stroma which probably have an important influence on the effect of the stimulating substances which are brought to the tissues

9. Blumenthal, H. T., and Loeb, L.: Am. J. Path. 18:615, 1942.

10. Dosne, C., and Dalton, A. J.: Anat. Rec. 80:211, 1941.

11. Selye, H.: Endocrinology 21:169, 1937.

12. Sarason, E. L.: Arch. Path. 35:373, 1943.

13. Evans, G. T.: J. Biol. Chem. 35:105, 1934; Am. J. Physiol. 114:297, 1936.

14. Lewis, R. A.; Thorn, G. W.; Koepf, G. F., and Dorrance, S. D.: J. Clin. Investigation 21:33, 1942.

15. Flexner, L. B., and Grollman, A.: Anat. Rec. 75:207, 1939.

16. Whitehead, R.: J. Anat. 69:72, 1934.

as well as on the removal of metabolic products from the tissues. These factors have been discussed by Loeb, and the present experiments serve to substantiate his observations in various inbred strains of mice. The method of determining mitotic activity which was used here lends itself well to a quantitative analysis of the changes in growth processes which occur with aging. Donaldson,[17] Korenchevsky [18] and others have attempted to analyze the gradual decrease in growth of an organ by determining the decrease in weight of the organ per unit of body weight. However, as Korenchevsky pointed out, such a determination is "without the implication that atrophic changes also occur in the cells of that organ." Furthermore, I [19] have previously shown that in the case of the adrenal gland of the guinea pig the determination of the changes in the weight of the gland is not an accurate means of demonstrating its proliferative activity; this probably also holds true for other organs.

SUMMARY

In these experiments the changes in the proliferative activity of the thyroid, parathyroid and adrenal glands of the guinea pig under various conditions were determined quantitatively by counting the mitoses in these organs. A gradual diminution in the number of mitoses was found in all three of these glands with increasing age. The greatest diminution in the number of mitoses occurred in the transition from embryonic life to the earliest period of extrauterine life, after which the difference between the mitotic activity in two successive periods gradually diminished. With the exception of the embryonic organs, in which a certain variability exists in this regard, the average mitotic activity in these three organs is greater in female guinea pigs than in males of a corresponding age, and the ratio of mitotic activity in males to that in females remains almost constant in all age groups.

The stroma of these glands changes with increasing age from a loose fibrillar to a hyaline-fibrous tissue. In the adrenal gland an inverse relationship was found between the degree of mitotic activity and the number of vacuoles in the zona fasciculata. In accordance with our previous findings there is some indication that this structural difference in the adrenal gland may be causally connected with the differences in mitotic activity between the two sexes, with the differences observed during pregnancy and with the diminution with advancing age.

17. Donaldson, H. H.: The Rat, American Anatomical Memoir 6, Philadelphia, Wistar Institute of Anatomy and Biology, 1925.

18. Korenchevsky, V.: J. Path. & Bact. **54**:13, 1942.

19. Blumenthal, H. T.: Endocrinology **27**:486, 1940.

REGENERATION OF EPIDERMIS OF MICE UNDER THE INFLUENCE OF 20-METHYLCHOLANTHRENE

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Painting of the skin of mice with benzpyrene or with 20-methylcholanthrene for a varying length of time previous to the making of a wound stimulated the proliferation of the regenerating epithelium but did not accelerate epithelization of the defect at a corresponding rate.[1] In an attempt to interpret this result, the following possibilities were considered: (1) The epithelial cells might be directly injured by the carcinogen; (2) the intensified keratinization of the spinous cells caused by the carcinogen might interfere with the motility of the epidermal cells; (3) changes produced by the carcinogen in the base of the wound might deprive the epithelial cells of the solid base necessary for stereotropic movement.

In order to analyze further the influence of carcinogenic agents on epithelial growth and movement, we studied the regeneration of the epidermis of mice after 20-methylcholanthrene had been applied directly to a wound and to the adjoining tissue.

MATERIAL AND METHODS

Twenty-eight white Swiss mice 6 to 8 weeks old and maintained on a diet of Purina Chow and water were used. The hair of the back was clipped; a circular piece of epidermis together with some of the underlying connective tissue measuring 7 mm. in diameter was excised with a pair of curved scissors. Healing was allowed to take place for three, five, eight, eleven, fourteen, twenty-one and twenty-eight days.

The animals were divided into three groups, with litter mates evenly distributed as far as possible.

Series 1 (7 mice, untreated).—Regeneration took place without further experimental interference.

Series 2 (7 mice, benzene treated).—The wounds and the surrounding epidermis were painted three times every week with benzene, which was applied with one stroke of a camel hair brush, no. 6, the first treatment being given immediately after excision.

1. Silberberg, M., and Silberberg, R.: (a) Am. J. Path. **20**:809, 1944; (b) Arch. Path. **38**:215, 1944; (c) **39**:257, 1945. (d) Dietrich, D.: Ztschr. f. Krebsforsch. **48**:187, 1938.

Series 3 (14 mice, methylcholanthrene treated.)—The wounds and the skin were painted with 0.3 per cent 20-methylcholanthrene dissolved in benzene. The carcinogen was applied in the same way and at the same intervals as benzene was in series 2.

At the end of an experimental period, the animals whose wounds were to be studied were killed between 10 and 11 a. m. Each wound with the adjoining skin was removed, stretched on filter paper, fixed in 10 per cent solution of formaldehyde, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

HISTOLOGIC EXAMINATION

The histologic observations are presented in tables 1 to 3. The number of cell rows in the epithelium was determined at some distance from the wound (column 4), at its margin (column 5) and in the regenerating epithelial tongue (column 6); the lengths of the tongues are given in column 7. The ratio between basal and spinous cells was determined (column 3). As in previous investigations,[1b] the number of mitoses is given in multiples of the normal, which in this strain of mice is 12 mitoses in 10,000 basal cells. The results of the mitotic counts in the original epithelium appear in column 8, and those in the new epithelium in column 9; the mean values and the maximum and minimum deviations are indicated.

1. *Untreated Series.*—(a) Old Epithelium: Throughout the period of observation, the epidermis at some distance from the wound consisted of the usual two rows of epithelial cells, and the ratio of basal to spinous cells ranged from 2.2:1 to 2.7:1. The epidermis at the margin of the defect consisted of five or six layers of cells three and five days after operation; a maximum thickness of six or seven cell rows was reached eight days after excision. After twenty-one and twenty-eight days, only four or three cell rows were counted, which is slightly more than the ordinary number of two. Corresponding to the increased thickness of the epidermis there was an increase in the number and the size of the epithelial cells, reaching a maximum eight days after the making of the wound. The mean mitotic count was three times the normal three days after operation; five days after operation it reached a peak of six times the normal; subsequently it dropped to three or four times the normal, and fourteen days after excision, coinciding with the closure of the wound, a return to the usual figure had occurred.

(b) New Epithelium: The tongues advancing toward the center of the wounds increased in length from 0.29 mm. at three days to 0.91 mm. at eleven days after excision of the tissues; fourteen days after this operation they had met and bridged the defect. They showed from three to five layers of cells during the first eleven days of observation; subsequently the number of cell rows equaled that in the marginal epithelium. The migrating

cells were about 10 per cent larger than the epithelial cells at the margin of the wound. Whereas three days after excision mitoses were scarce, showing a mean of one sixth of the normal, they increased markedly at later stages, reaching a maximum mean of six times the normal five days after the making of the wound. Thereafter their number dropped gradually from a mean of four times the normal eight days after excision to three times the normal fourteen days after excision. After twenty-one and twenty-eight days the mitotic count was normal again.

2. *Benzene-Treated Series.*—(*a*) Old Epithelium: The epidermis was thicker and more keratinized than ordi-

mitoses in a given area had fallen to a mean of four and five times the normal, and after eleven days, coinciding with the closure of the wound, another steep drop to one and a half times the normal indicated the return to the resting state. This compares with a mean maximum of six times seen after five days and a return to normal observed after fourteen days in the untreated mice.

(*b*) New Epithelium: The regenerating tongues were 0.37 mm. long after three days of observation (0.29 mm. in the untreated series), and they measured 0.56 mm. after five days (0.41 mm. in the control group). The tongues met after eight days, whereas in untreated mice

TABLE 1.—*Untreated Animals*

| Mouse | Days of Observation | Ratio Basal : Spinous Cells | Cell Rows in | | | Length of Tongue, Mm. | Mitoses Expressed in Multiples of the Normal | |
			Distant Epithelium	Marginal Epithelium	Tongue		Original Epithelium *	New Epithelium *
319	3	2.5:1	2	5-6	3-4	0.29	3 max. 4½ min. 2	⅙ max. ¼ min. 0
315	5	2.7:1	2	5-6	3-4	0.41	6 max. 8 min. 4½	6 max. 8 min. 5
311	8	2.5:1	2	6-7	4-5	0.57	3 max. 4 min. 2	4 max. 5 min. 3
309	11	2.5:1	2	4-5	3-4	0.91	4 max. 5 min. 3	3½ max. 4 min. 2
303	14	2.2:1	2	5-6	..	Closing	1 max. 1½ min. ¾	3 max. 5 min. 2¼
299	21	2.5:1	2	3-4	..	Closed	1 max. 1½ min. ¼	1 max. 1½ min. ¼
295	28	2.5:1	2	3	..	Closed	1 max. 1½ min. ½	1 max. 1 min. 1

* The first figure represents the mean. The maximum and the minimum deviation are given to the right of the mean.

TABLE 2.—*Benzene-Painted Animals*

| Mouse | Days of Observation | Ratio Basal : Spinous Cells | Cell Rows in | | | Length of Tongue, Mm. | Mitoses Expressed in Multiples of the Normal | |
			Distant Epithelium	Marginal Epithelium	Tongue		Original Epithelium *	New Epithelium *
320	3	2.7:1	2-3	6-7	4	0.37	9 max. 11 min. 8	3½ max. 5 min. 2¼
316	5	2.2:1	3	6-7	4	0.56	5 max. 7 min. 3½	6 max. 9 min. 5
312	8	2:1	3	6-7	5	Closing	4 max. 5 min. 3	5½ max. 7 min. 4
308	11	1.5:1	3-4	5-6	..	Closed	1½ max. 2 min. 1	2 max. 3 min. 1
304	14	1.7:1	3-4	5-6	..	Closed	1 max. 1½ min. ½	¼ max. 1 min. ¼
300	21	1.5:1	4	3-4	..	Closed	1 max. 1 min. 1	1 max. 1 min. 1
296	28	2:1	3	4	..	Closed	1½ max. 1½ min. 1	1½ max. 1½ min. 1

* The first figure represents the mean. The maximum and the minimum deviation are given to the right of the mean.

narily, showing its greatest thickness of three or four cell layers after eleven to twenty-one days of painting. At the same time, the number of spinous cells increased and the ratio of basal to spinous cells rose from 2.7:1 to 1.7:1 and 1.5:1; after twenty-eight days of observation the ratio was 2:1 again.

Three and five days after excision, six or seven cell layers were counted at the margin of the wound as against five or six in untreated animals; subsequently the conditions were similar to those found in the control series. During the first week of painting, the cells at the margin of the defect were 10 per cent larger than those in the nontreated animals. The number of mitoses rose markedly as early as after three days, when the mean mitotic count had reached its peak of nine times the normal. After five and eight days the number of

this occurred only after fourteen days. The number of cell layers in the tongues did not differ significantly from that in the untreated series. However, under the influence of benzene, the epithelial cells in the tongues were larger than those in the untreated mice.

The number of mitoses increased to a mean of three and a half times the normal three days after the making of the wound, while that in the untreated animals was much below normal. After five days the mean mitotic count was six times the normal in both groups. After eleven and fourteen days the number of mitoses in the benzene-treated mice was twice the normal or normal, whereas in the untreated mice the mitoses were still three or three and a half times more numerous than ordinarily. Thus under the influence of benzene the drop of the mitotic count occurred at an earlier date

and was steeper. This is in accordance with the earlier closure of the wound after application of benzene.

3. *Methylcholanthrene-Treated Series.*—(*a*) Old Epithelium: With increasing duration of the treatment with methylcholanthrene, the epidermis at some distance from the line of excision underwent pronounced thickening and keratinization, and after twenty-one and twenty-eight days of treatment it consisted of from four to six cell layers. Under the influence of benzene alone, there were three or four cell rows. During the first eleven days of application of methylcholanthrene the epidermal cells were 10 to 15 per cent larger than after treatment with benzene alone. Subsequently, however, the hypertrophy was less marked in both groups. The ratio of basal to spinous cells increasingly favored the latter, changing from 2.2 :1 or 2.5 :1 after three days to between 1.5 :1

diminish after five or eight days and dropped to normal or almost normal from the eleventh day on.

(*b*) New Epithelium: The epithelial tongues measured 0.38 mm. (mean) after three days of treatment and 0.61 mm. (mean) after five days. In the benzene-treated series the corresponding figures were 0.37 mm. and 0.56 mm. After eight days' treatment with the carcinogen in 1 animal the tongues had joined, and after eleven days they had met in both mice as they had in the benzene-treated animals. The tongues showed a similar thickness in both groups. However, during the first eleven days of treatment with methylcholanthrene the advancing epithelial cells were somewhat larger than after application of benzene.

After three days of regeneration the mean mitotic count in the methylcholanthrene-treated mice was three

TABLE 3.—*Methylcholanthrene-Treated Animals*

Mouse	Days of Observation	Ratio Basal : Spinous Cells	Cell Rows in Distant Epithelium	Cell Rows in Marginal Epithelium	Cell Rows in Tongue	Length of Tongue, Mm.	Original Epithelium	Mean	New Epithelium	Mean
321	3	2.2:1	2-3	6	3-4	0.40	9 max. 10 min. 7	8	2 max. 4½ min. 2½	3
329	3	2.5:1	2-3	4-5	3-4	0.36	7 max. 9 min. 6		4 max. 5 min. 2½	
317	5	2:1	3-4	7-8	4-5	0.63	8 max. 10 min. 7	8½	6 max. 7 min. 4½	7
318	5	1.7:1	3-4	6	3-4	0.59	9 max. 11 min. 7		8 max. 9 min. 7	
313	8	1.5:1	4-5	9-10	6-7	Closing	6½ max. 7 min. 5½	7½	8 max. 9 min. 6	8
314	8	1.5:1	5-6	7-8	4-5	0.94	8½ max. 10 min. 7		8 max. 9 min. 6	
307	11	1.3:1	4-5	8-9	4-5	Closed	8 max. 10 min. 7	8	5½ max. 6½ min. 5	7
310	11	1.5:1	4-5	6-7	...	Closed	8 max. 9 min. 7		8½ max. 10 min. 6	
305	14	1:1	6	5-6	...	Closed	7 max. 8 min. 6	6½	4 max. 5 min. 3	6
306	14	1.7:1	4-5	4-5	...	Closed	6 max. 7 min. 4		8 max. 9 min. 7	
301	21	1.5:1	5-6	4-5	...	Closed	6½ max. 8 min. 6	7¼	2½ max. 3½ min. 2	2
302	21	1.7:1	5	5	...	Closed	8 max. 10 min. 6		1½ max. 2 min. 1	
297	28	1.2:1	4-5	4	...	Closed	8 max. 9 min. 7	8	3 max. 3 min. 3	3
296	28	1.3:1	5	4-5	...	Closed	8 max. 9 min. 7		3 max. 4 min. 2	

and 1 :1 after eight and more days; this shift was more accentuated and more persistent in this series than in the series painted with benzene. The number of cell layers at the margin of the defect rose from four to six after three days to seven to ten after eight days. After treatment with benzene alone, the maximum thickness of six or seven cell layers was found from three to eight days after the wound was made. After fourteen days of application of methylcholanthrene the number of cell rows fell to four or six and after twenty-one and twenty-eight days the number of cell layers at the margin of the wounds was still somewhat greater in the methylcholanthrene-treated animals than in those painted with benzene.

After three days of application of methylcholanthrene the mean mitotic count was eight times the normal as against nine times the normal in the benzene-treated animals. It remained at about the same height (eight times the normal) throughout the duration of the experiment, whereas under the influence of benzene it began to

times the normal. After five days it rose to seven times the normal (six times in the benzene-treated animals), and after eight days the corresponding values were eight for the methylcholanthrene-treated but only five and a half for the benzene-treated mice. Whereas in the latter a marked fall occurred after eleven days and a return to normal after fourteen days, in the methylcholanthrene-painted mice the mitotic count still showed a mean of six times the normal at this stage. After twenty-one or twenty-eight days a decline to twice or three times the normal took place. The new epithelium thus differed from the old, in which the mitotic count remained high throughout the period of observation.

COMMENT

Methylcholanthrene applied to experimental wounds accelerated regeneration of the skin of mice. Chart 1 *A* demonstrates the mitotic cycle

in the original epithelium during four weeks of observation in (1) untreated, (2) benzene-painted and (3) methylcholanthrene-treated wounds; chart 1 *B* shows the corresponding cycles in the new epithelium.

During the first stages of repair the rate of proliferation, as well as that of migration, of the epithelial cells was influenced similarly by methylcholanthrene and benzene; but whereas in the benzene-treated animals the mitotic count dropped

Chart 1.—*A*, mitotic cycle in the original epithelium during twenty-eight days of wound repair in control (untreated) mice (——) and in mice treated with benzene (. . . . .) and mice treated with methylcholanthrene (- - - - -) for twenty-eight days. *N* stands for the normal value and the numbers above *N* for multiples of the normal value. *B*, mitotic cycle in the new epithelium in the same groups of animals.

quickly, in the methylcholanthrene-painted mice it remained at a fairly steady high level even after epithelization of the wounds was completed. The decline of mitotic proliferation ordinarily occurring at this time evidently was prevented by the carcinogen. Likewise, in the new epithelium from eight days on after the beginning of the experiment the growth processes were more accentuated after treatment with methylcholanthrene than after treatment with benzene alone. However, in spite of this increased proliferation, the migration of the epithelium over the wounds progressed only at about the same rate as under the influence of benzene (tables 2 and 3). After the defects had been covered by regenerating epidermal cells, the number of mitoses in the new epithelium fell to a point considerably lower than that in the old epithelium. This observation suggests that the new epithelium was more strongly affected by the meeting of the epithelial tongues than was the old. However, as compared with conditions in untreated skin, the decline of the number of mitoses was definitely delayed; it did not coincide with the closure of the wounds

but was noted about one week afterward. After twenty-eight days of treatment with the carcinogen, a slight rise of the mitotic count was found in the new epithelium. If this is not merely a chance phenomenon, it may indicate an increasing responsiveness to methylcholanthrene.

Chart 2 makes possible a comparison of the mitotic cycles after application of benzpyrene and methylcholanthrene, respectively. The two carcinogens stimulated the mitotic activity in the old epithelium similarly during the first eleven days, but from then on methylcholanthrene seemed to be somewhat more potent (chart 2 *A*). In neither group did the migration of the epithelium into and the closing of the wounds proceed at a rate commensurate with the increase in proliferative activity. However, the dissociation of cell proliferation and migration was more marked in the benzpyrene series. Moreover, as indicated by the number of cell layers, the epidermis was thicker after application of methylcholanthrene than after that of benzpyrene. Both these differences might be due to the fact that methylcholanthrene causes less keratinization than benzpyrene. Less keratinization, however, means the preservation of more of the superficial layers of the epidermis. The diminished keratinization in the methylcholanthrene-treated animals may allow cell migration to proceed in a more normal
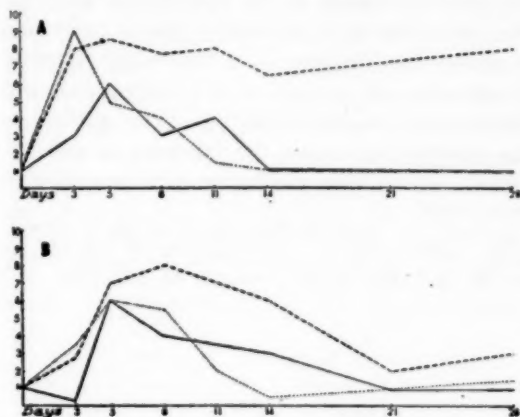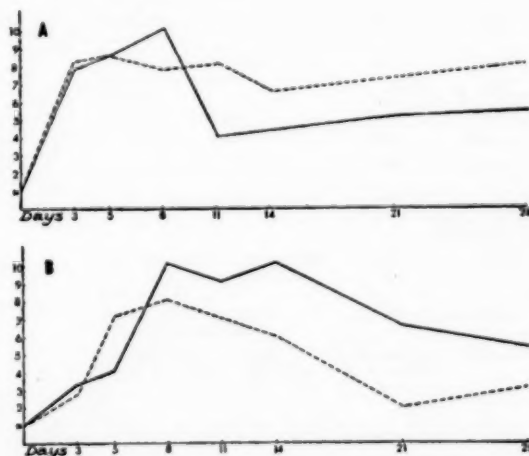

Chart 2.—*A*, mitotic cycle in the original epithelium during twenty-eight days of wound repair in mice treated with benzpyrene (——) and in mice treated with methylcholanthrene (- - - - -). *N* stands for the normal value and the numbers above *N* for multiples of the normal value. *B*, mitotic cycle in the new epithelium in the same groups of animals.

manner than it does under the influence of benzpyrene. This correlation between the differences in cell migration, on the one hand, and the variations of keratinization, on the other, seems to support our previous suggestion concerning the

adverse effect of the latter on epithelial motility. This, however, does not exclude the possibility that changes in the wound base, particularly in its contractibility, play a role in the comparatively slow closure of the wounds.

In the new epithelium (chart 2 B) the mitotic curve took a more normal course under the influence of methylcholanthrene than after painting with benzpyrene. In the experiments with the latter the mitotic cycle showed a plateau between five and fourteen days after excision, whereas in those with methylcholanthrene it began to decline after reaching a peak at eight days. This may be correlated with the different rates at which the wounds closed. The wounds in the methylcholanthrene group were closed eight or eleven days subsequent to the excision, but those under the influence of benzpyrene were still open at this time. As Loeb [2] has shown, the closing of the wound is soon followed by a marked fall in the mitotic activity of the new epithelium. Corre-spondingly, a larger skin defect offers a stronger stimulus to growth than a smaller one. In the benzpyrene series the stimulus produced by the wound lasted longer and thus kept the mitotic count at a higher level.

SUMMARY

Methylcholanthrene applied to regenerating epidermis of mice increases the proliferation and accelerates the migration of the epithelium. However, the movement of the epithelium over the defect proceeds at a relatively slower rate than the mitotic multiplication of the cells. Neither the dissociation of epithelial proliferation and migration nor the keratinization of the epithelium is as conspicuous under the influence of methylcholanthrene as after treatment with benzpyrene.

2. Loeb, L.: Arch. f. Entwcklngsmechn. d. Organ. 24:638, 1907. Loeb, L., and Spain, K. C.: J. Exper. Med. 21:193, 1915; J. M. Research 41:247, 1919.

MICROFILARIAL GRANULOMA OF THE SPLEEN

OBSERVATIONS IN TWELVE ADDITIONAL CASES

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In a previous communication [1] a report was made of a study of specimens of 11 spleens in which multiple, well circumscribed, dark red, nodules, varying in size from 2 mm. to 5 cm., were encountered. Histologic examination showed in many of the nodules an eosinophilic cell infiltration and in every one of them a chronic inflammatory reaction suggestive of a granulomatous lesion. Microscopically, each nodule showed microfilarias cut in various planes. From this evidence it was suggested that the lesions were of the nature of microfilarial granuloma.

macroscopic features of the typical lesions were so characteristic that in every one of the 12 cases a confident diagnosis as regards the nature of the nodules was made on inspection of the spleen, and subsequently it became possible to demonstrate the living microfilarias in all except 2 cases, in which the spleen was in the atrophic state and the nodules were fibrotic (table 2).

GENERAL OBSERVATIONS

Table 1 gives the age and the sex of the patient, the duration of stay in the hospital, the clinical diagnosis,

TABLE 1.—*Details of Twelve Additional Cases of Microfilarial Granuloma of the Spleen*

| Case	Year	Sex	Age	Duration of Hospitalization	Clinical Diagnosis	Postmortem Diagnosis	Weight of Spleen, Gm.	Filaria (Adult Worm)
12	1941	M	35	Few minutes	Fracture of femur	Fracture of femur and ribs, shock	115	Present
13	1941	M	25	3 hours	Injury of head	Depressed fracture of skull, laceration of brain	220	Absent
14	1941	F	70	2 days	Fracture of tibia and fibula	Toxemia due to suppuration at site of fracture	125	Absent
15	1941	M	35	Few hours	Injury of head	Fracture of base of skull, cerebral hemorrhage	225	Absent
16	1941	M	50	Few hours	Pneumonia	Congestion, cardiac failure due to chronic bronchitis	160	Absent
17	1941	F	46	3½ hours	?	Septic peritonitis	110	Absent
18	1941	M	35	3½ hours	Gastroenteritis	Bronchopneumonia	230	Absent
19	1942	M	25	2 hours	Injury of head, depressed fracture	Cerebral hemorrhage	190	Absent
20	1942	M	45	2 hours	?	Opium poisoning	205	Absent (microfilaris in the epididymis)
21	1942	M	32	2 hours	Stab wound of abdomen	Multiple perforation in the intestines, hemorrhage, shock	105	Absent
22	1943	M	35	18 hours	Burns	Burns and mydriatic alkaloid poisoning	185	Absent
23	1943	M	30	8 hours	?	Thrombosis and gangrene of mesenteric vein	2½ times the normal	Absent

During the course of the years 1941 to 1943 specimens of 12 more spleens, making a total of 23, bearing these nodules came under observation. It is the purpose of this article to extend the observations previously made from the material which has subsequently become available for study.

I should like to reiterate in regard to the later material the observation previously made: The

the diagnosis arrived at in the postmortem examination, the weight of the spleen and the presence or the absence of the adult filarial worm. From this table it may be seen that the age incidence varied from 25 to 70 years. Included in the previous observations [1] were 2 spleens of children 6 and 8 years old. Twenty of the 23 patients observed so far were between 25 and 50 years of age. There were 5 females and 18 males. The duration of hospitalization varied from a few hours to two days. In 1 case it was only a few minutes. This is in conformity with the previous observation that the nodules are encountered in patients who have died early in the course of illness. It will be seen from the clinical diagnosis that a majority of these patients had undergone trauma, such as an injury of the head or multiple fractures, as a result of which they died early. The
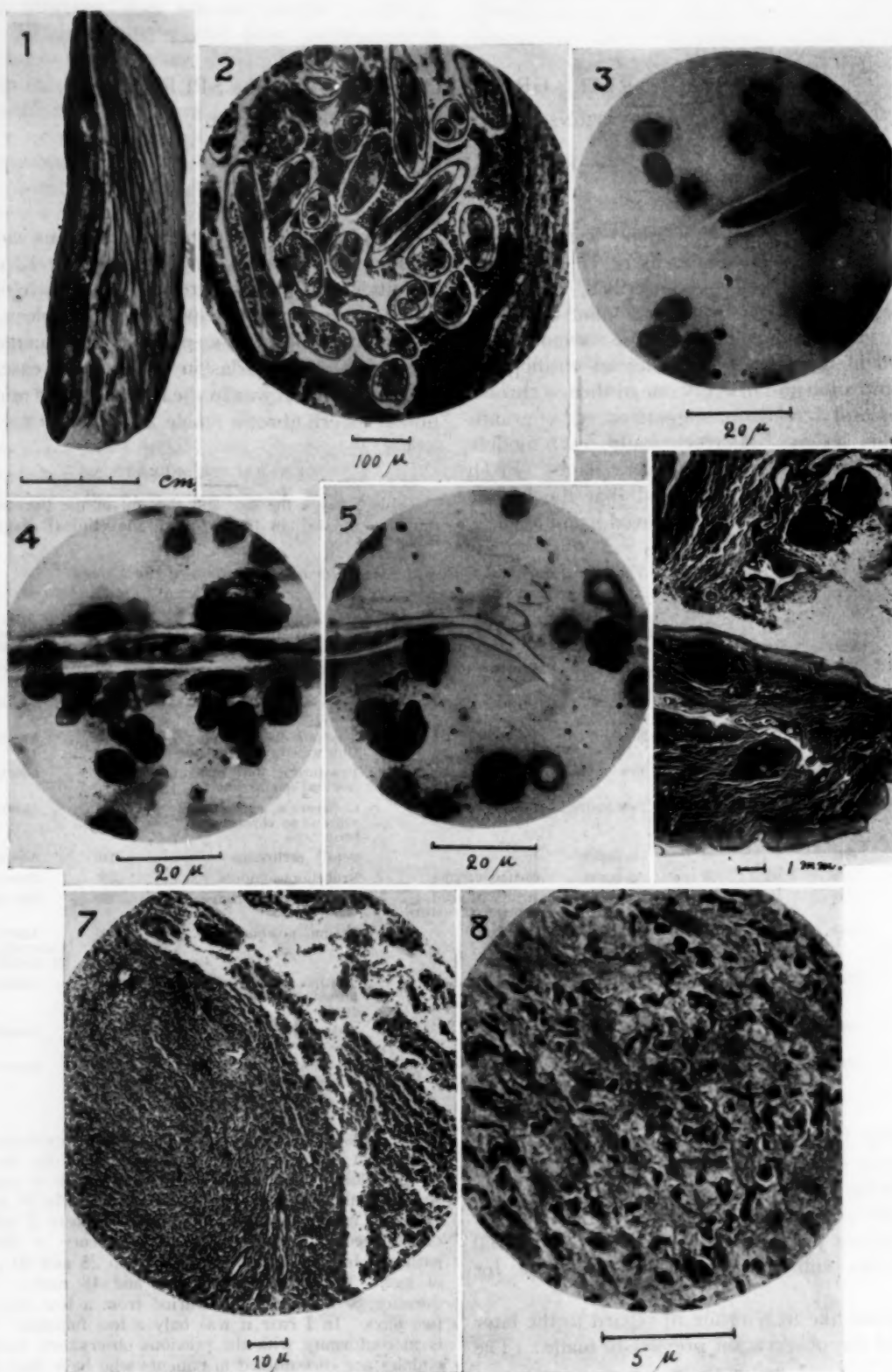
(See legends on opposite page)

indications are that before death each had been in a relatively healthy condition.

In the previous communication the remark was made that the weight of the spleen was not altered in cases of microfilarial granuloma. In the present group, in 7 cases the weight of the spleen was more than 180 Gm., which may be taken as the average normal weight of the spleen. It must be pointed out that the increase of 40 to 50 Gm. in weight might be accounted for by old malarial infection, which is common in this country. In 1 case in this series an adult female filarial worm was seen in the epididymis, and in another segments of microfilarias were seen in the same organ.

REPORT OF A CASE IN DETAIL

Case 12 in which the adult filarial worm was seen is remarkable and merits a more detailed description.

A man aged 35 was admitted with the history that he was hurt by a falling tree. A femur was fractured, and surgical emphysema was detected involving the chest and the upper extremities. He succumbed in the hospital within a few minutes after his admission. At the autopsy microfilarial nodules were detected in the spleen (fig. 1). Material scraped from the nodule showed living microfilarias in much larger number than material scraped out of the neighboring normal splenic tissue. A drop of blood was taken from cardiac blood, and it also showed microfilarias. On further examination it was found that the testis and the epididymis on one side were slightly bigger than those on the other. On palpation a small encapsulated nodule (1 cm. in diameter) was felt in the epididymis. Shining through the capsule were coils of a threadlike worm. It was difficult to extricate it out of the mass. It was therefore transferred for histologic examination, and cut portions (fig. 2) showed that it was a female filarial worm, the uterus of which with a number of ensheathed microfilarias was seen in a good state of preservation. The nodule had an inflammatory reaction around it, but the cut segments of the worm appeared healthy, i. e., without evidence of degeneration or calcification. This indicated that the worm was probably in a living condition in the body. It might be noted that the sections taken from the lung showed microfilarias lying in the lumens of the blood vessels. Mention must be made of the fact that material taken out of the marrow of the femur did not show microfilarias.

The Microfilarias.—In the study previously reported [1] the available material was all fixed tissues. From the postmortem material dealt with in the present report, living specimens of microfilarias were obtained for a detailed study of the characters of microfilarias. It is well recognized that two types of microfilarias are found in the blood of persons suffering from filarial infection in India, viz., those of the Wuchereria bancrofti and those of the Wuchereria malayi type. These two types can be differentiated, one from the other, by their microscopic features. A critical study of the morphologic features of microfilarias may be made from the photomicrographs in figures 3, 4 and 5, which show them as viewed with the oil immersion lens. The following points can be readily made out: 1. The nuclei are well outlined and spaced and can be counted without difficulty. 2. The cephalic space is as long as it is broad. 3. The tail end tapers to a point, and the terminal portion contains no nuclei. 4. The excretory pore and the cell lie close together, and a thread of protoplasm runs posteriorly from the latter, although this point is not so well brought out in the photomicrographs. These features are sufficient to indicate that the microfilarias in question belong to the Bancroft variety and not to the Malayan, in which the nuclei are blurred and intermingled and therefore cannot be easily counted. In the latter the tail tapers to a point, which is continued as a fine thread. The tapered portion contains one nucleus, while in the thread two nuclei are seen. The cephalic space is twice as long as it is broad and the excretory pore and the cell are separated.

Adult Female Filaria.—In addition to the microscopic features, an important confirmatory point is the occurrence of the adult female filaria, which was found in the epididymis in this case. It is well recognized with respect to W. malayi that only the embryonic form, or the microfilaria, is known; the adults have not yet been identified. With this observation and from the evidence cited in the foregoing section it is concluded that the variety of Filaria responsible for the nodules is Wuchereria bancrofti.

FIBROSIS OF NODULES

Table 2 gives the details of 2 cases in which the spleen was in an atrophic condition and showed on minute inspection tiny nodules about 2 mm. in diameter (fig. 6). Material scraped from them did not show any microfilarias nor was it possible to demonstrate segments of microfilarias in cut sections from the nodules or other tissues given for section at autopsy. Histologically, the nodules were well circumscribed

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EXPLANATION OF PLATE

Fig. 1.—A photograph of a cut portion of the spleen in case 12 to show the size and the character of the nodules. Histoligic sections showed segments of microfilarias.

Fig. 2.—Section of the nodule in the epididymis in case 12 showing segments of an adult female filarial worm. The segments show cut portions of numerous microfilarias filling the uterine cavity of the worm.

Figs. 3, 4 and 5.—Photomicrographs of microfilarias obtained in a living condition by teasing the nodules. In figure 3 note that the cephalic space is as long as it is broad. In figure 4 it may be seen that the excretory pore and the cell lie close together. Figure 5 shows that the nuclei are well outlined and spaced and can be counted without difficulty. It also shows that the tail end tapers to a point and that the terminal portion contains no nuclei.

Fig. 6.—A low power picture of the nodules as they are seen in histologic preparations. Note that they are well circumscribed and of compact structure.

Fig. 7.—The peripheral portion of one of the nodules shown in figure 6; × 10. The splenic tissue at the periphery of the nodules is compressed. Note the dense structure of the nodules as compared with the surrounding renal parenchyma.

Fig. 8.—Evidence of fibrosis in the nodule. The cells with large nuclei are reticuloendothelial cells; those with elongated nuclei are proliferating fibroblasts.

(fig. 7) with a certain amount of compressed splenic tissue around them. The nodular tissue was denser than the splenic tissue. Malpighian bodies, trabeculae of septums or any large-sized blood vessels were not seen in them. They were made up of proliferated reticuloendothelial cells and fibroblasts (fig. 8). Sections stained with Van Gieson's stain showed a large amount of fibrous tissue. No giant cells were seen in their structure.

From the foregoing description it may be easily seen that these nodules have the same structure as those in which microfilarial segments were seen, except that the early granulomatous reaction, characterized by the presence of lymphocytes, eosinophilic cells or giant cells, was not seen in them. From their general features it might be stated that they probably represent a fibrosed condition of a granulomatous lesion from which the early inflammatory reaction, together with the segments of microfilarias, has

whether this is just an accidental finding or whether the two bear a causal relation to each other. The association has been observed in too large a number of cases for one to dispose of it lightly as accidental. It would be interesting to discuss what causal relationship could exist between the two. One explanation might be that trauma somehow releases a large number of microfilarias, which flood the circulation, and that the nodules represent one of the methods by which the dead or degenerating larvae are delimited and ultimately disposed of. In favor of this view are the well circumscribed nature of the lesions, the concentration of the larvae in the nodules and the inflammatory character of the tissue reaction in them. However, in many of these cases this view is rendered improbable by the short interval between the trauma and the death of the patient. It is difficult to conceive that a chronic inflammatory reaction of the nature seen in sections of these nodules could be brought

TABLE 2.—*Details of Two Cases in Which Fibrosed Nodules Were Seen*

Case	Year	Sex	Age	Duration of Hospitalization	Clinical Diagnosis	Postmortem Diagnosis	Weight of Spleen	Filaria
1	1943	M	50	2½ hours	Compound fracture of tibia and fibula	Shock and multiple injury	Small size	Absent
2	1943	F	50	12 hours	Intracranial hemorrhage	Fracture of skull, cerebral hemorrhage, laceration of brain	65 Gm.	Absent

disappeared, leaving a nodule which might ultimately result in scar formation. The fact that in both of these cases the spleen was in an atrophic state lends further support to this view. This finding is in conformity with the view expressed in the previous communication that the granulomas would gradually become sclerosed, ultimately resulting in tiny or insignificant scars.

COMMENT

The macroscopic features of the microfilarial granuloma are so well defined that by mere inspection it is possible to say that the lesion is microfilarial. The histologic features are also characteristic, so that under the microscope the granuloma is typical of this condition and is not seen in any other parasitic or bacterial infection. The presence of microfilarias either in a living or in a degenerate condition in the unfibrosed nodules is so constant as to justify the conclusion that these lesions are produced by them.

One of the intriguing observations that have been made is that trauma is associated with the nodules in the spleen. The question arises as to

about in such a short space of time. The other possibility is that persons who are parasitized are likely to suffer from accidents more than those who are free from parasites. It is only recently that people are realizing the part played by organic disease in the psychic and mental inefficiency of those suffering from such diseases. These two views indicate the lines along which further work to elucidate this problem might be carried on. At present enough information has not accumulated to give a considered opinion in regard to the curious association of injury with the lesions seen in these cases.

SUMMARY

The evidence from 12 specimens of spleens containing microfilarial nodules shows that the microfilarias are of the Wuchereria bancrofti type. In 1 case an adult female filarial worm was found in the epididymis along with nodules in the spleen and microfilarias in the blood.

In fibrosed nodules seen in specimens of 2 spleens microfilarial segments were not observed.

The relationship of traumatic injury to the production of the nodules invites investigation.

Case Reports

BILATERAL OVARIAN CARCINOMA IN A THIRTY WEEK FETUS

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Carcinoma occurring in fetal life has not been found mentioned in the available literature. Morehead [1] in a recent paper described 7 cases in which carcinoma was observed in a young person. The 7 patients ranged from 13 to 29 years of age.

Scheffy and Crawford [2] reported a case of carcinoma of the cervix uteri of a 22 month old child.

Bowing and McCullough [3] collected 12 cases of cervical carcinoma occurring before the age of 20 years, and Glass [4] and Shaw [5] each reported a case.

Scattered cases of carcinoma of the uterine fundus of the young person have been reported. [6]

Primary carcinoma of the liver is said to have been seen in newborn infants. [1] Steiner [7] collected 75 cases of hepatic carcinoma occurring in a child. Fifty-three per cent of the patients were under the age of 2 years.

Adult teratoma of the ovary is rare in childhood and has not occurred as a congenital growth. [8] Embryonal teratoma tends to appear at an earlier age than the adult type but is considerably more infrequent than the adult teratoma. [8]

Batchelor and Maun [9] recently listed 63 cases of congenital tumor of the heart; most of the patients were newborn or older infants. Many of these patients showed other tumors, cysts and congenital anomalies, including tumors of the skin, the liver, the kidney, the breast and the brain.

That neuroblastoma and embryonal adenosarcoma (Wilms's tumor), as well as miscellaneous types of sarcoma and lymphoma, may occur in infants and young children is well known. It would seem unnecessary in this paper to mention all the cases in which a cancer was reported to have occurred in infancy and childhood.

According to Novak, [10] dysgerminoma is a common tumor of early life. It is found in children before puberty and also in adolescent persons. In his series of 17 cases, the youngest patient was 6 years of age.

According to Ewing, [11] there have been cases of granulosa cell tumor occurring in infancy, and dysgerminoma occurs "at rather early ages." Granulosa cell tumor of the ovary has occasionally occurred in childhood and in about one third of the 200 published cases of dysgerminoma the tumor has been observed in a child. [8] Most other ovarian tumors occur during or after the reproductive period of life and are rare in childhood.

Although there are abundant reports in the literature of tumors that occurred in infancy and childhood, no reports have been found of such tumors occurring in fetal life. It is believed, therefore, that the case to be reported is of some interest. In addition, the carcinoma itself was peculiar because it appeared to be a dysgerminoma arising from granulosa cells.

REPORT OF A CASE

The mother of the patient was a 20 year old white primipara, a housewife and typist. Her father is living and well. Her mother died in childbirth. She has three siblings living and well. One brother died of an unknown cause at the age of 18. She underwent appendectomy in 1942 and tonsillectomy in 1943. There is no family history of carcinoma, tuberculosis, diabetes or allergy.

The pregnancy was uneventful until the occurrence of hemorrhage due to placenta previa marginalis, followed by a breech extraction delivery of a stillborn premature baby on July 8, 1944.

Autopsy (July 10, 1944).—The white female fetus weighed 1,340 Gm. (2 pounds 14 ounces) and was estimated to have had a fetal life of thirty weeks. It was well developed. The extremities were severely cyanosed. The lobes of the lungs sank in water and showed no gross evidence of aeration. They were firm and purplish red. In numerous viscera, especially in the epicardium, the pleurae and the adrenal glands, there were petechial hemorrhages and small ecchymoses. No developmental defects or anomalies were observed. There was no evidence of erythroblastosis fetalis, and there were no gross indications of cancer. The ovaries, however, were slightly enlarged and grayish pink. They aroused no special interest at autopsy. Tissues were removed and sectioned routinely. The diagnosis based on the gross observations was prematurity and pulmonary atelectasis.

Histologic Examination.—There was some postmortem decomposition, especially marked in the adrenal glands. Considerable hemopoiesis was noted in the

From the Pathological Laboratory, St. Luke's Hospital.

1. Morehead, R. P.: Arch. Path. **38**:141, 1944.
2. Scheffy, L. C., and Crawford, B. L.: Am. J. Obst. & Gynec. **24**:118, 1932.
3. Bowing, H. H., and McCullough, J. A. L.: Am. J. Roentgenol. **45**:819, 1941.
4. Glass, M.: Am. J. Obst. & Gynec. **26**:104, 1933.
5. Shaw, W. F.: J. Obst. & Gynaec. Brit. Emp. **48**:239, 1941.
6. Lockhart, H.A.: Am. J. Obst. & Gynec. **30**:76, 1935.
7. Steiner, M. M.: Am. J. Dis. Child. **55**:807, 1938.
8. Barzilai, G.: Atlas of Ovarian Tumors, New York, Grune & Stratton, Inc., 1943.
9. Batchelor, T. M., and Maun, M. E.: Arch. Path. **39**:67, 1945.

10. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1941.
11. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1942.

liver and the spleen. There were general vascular congestion and extensive interstitial hemorrhage, especially in the adrenal glands and the lungs. The latter showed the usual fetal condition. Other tissues were not noteworthy.

Sections of the ovaries showed that the entire substance of each gland was practically replaced by carcinomatous tissue. The cancer cells contained numerous mitotic figures. Scattered through the neoplasm were numerous primitive graafian follicles. In some places the granulosa cells seemed to show stages of transition into neoplastic cells. This gave the impression that

Material was submitted to the Tumor Registry, and the description of the growth was confirmed, but the exact classification was left unsettled. The following is quoted from Lucké [12]: "The material submitted has been reviewed by several officers at this laboratory and also by our resident consultants, Commander Shields Warren and Dr. Enrique Koppisch. The changes observed in the ovaries appear quite unique and have proved of unusual interest. The histologic pattern of a bilateral diffuse undifferentiated neoplasm, involving the stromal tissue and leaving almost unaffected the primordial follicles of the ovaries is perhaps
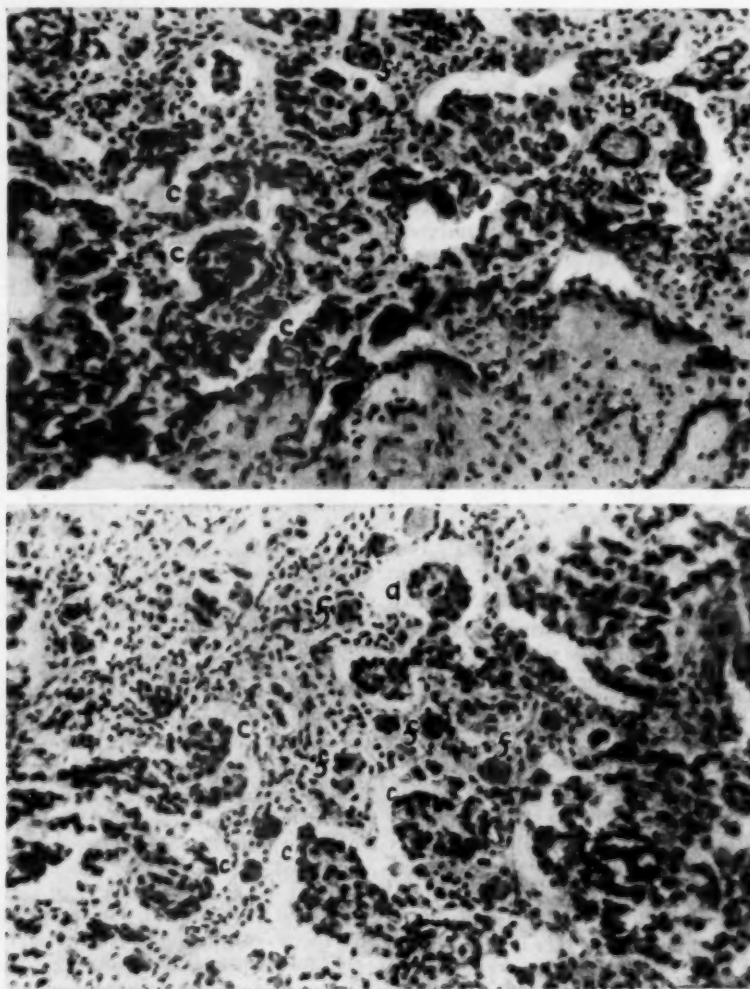

Fig. 1.—Granulosa cell tumor; × 250; hematoxylin and eosin stain: (*a*) Granulosa cells which have undergone neoplastic change; an ovum can be seen in the center. (*b*) Proliferating granulosa cells in an early stage of cancerous transformation. (*c*) Some other follicles which have become neoplastic. (*f*) Relatively normal primordial follicles.

the tumor was a granulosa cell carcinoma. However, the tumor cells themselves resembled those of dysgerminoma, and there was a delicate tumor stroma between the masses of tumor cells which also resembled the stroma of the well known dysgerminoma. There were no gross or microscopic evidences of extensions or metastases. This incidental observation of carcinoma of both ovaries of a fetus is probably without precedent. As far as can be determined, the carcinoma had no connection with the stillborn condition of the fetus.

the salient feature of this neoplasm. Some of those who studied these slides felt that the histologic characteristics of the tumor cells belong to the category of dysgerminoma. Others, including our resident consultant, Commander Shields Warren, thought they were able to trace transitions between the granulosa cell layer surrounding some of the ova and the tumor and that, therefore, the lesion might be classified as a granu-

12. Lucké, B.: Personal communication to the author, Nov. 1, 1944.

losa cell tumor. For the sake of our records we are classifying this lesion as 'carcinoma, undifferentiated, bilateral, congenital, of the ovaries.'"

The mother is reported at this time to be living and well. She has since (June 1945) given birth to a full term, well developed, normal-appearing infant.

COMMENT

It is clear from the studies cited in the foregoing pages that the exact classification of the tumor is a moot problem. From my study of the

lead to the discovery of other cases of new growth in fetal life.

According to Ewing,[11] the granulosa cell tumor is thought to originate from groups of embryonal cells in the region of the ovarian hilus as described in 1903 by Walthard. These cell groups, "granulosa ballen," are rarely found in the ovary after the first year of life. They are assumed to be superfluous cells which normally would develop into follicles. Von Werdt was the first to sug-
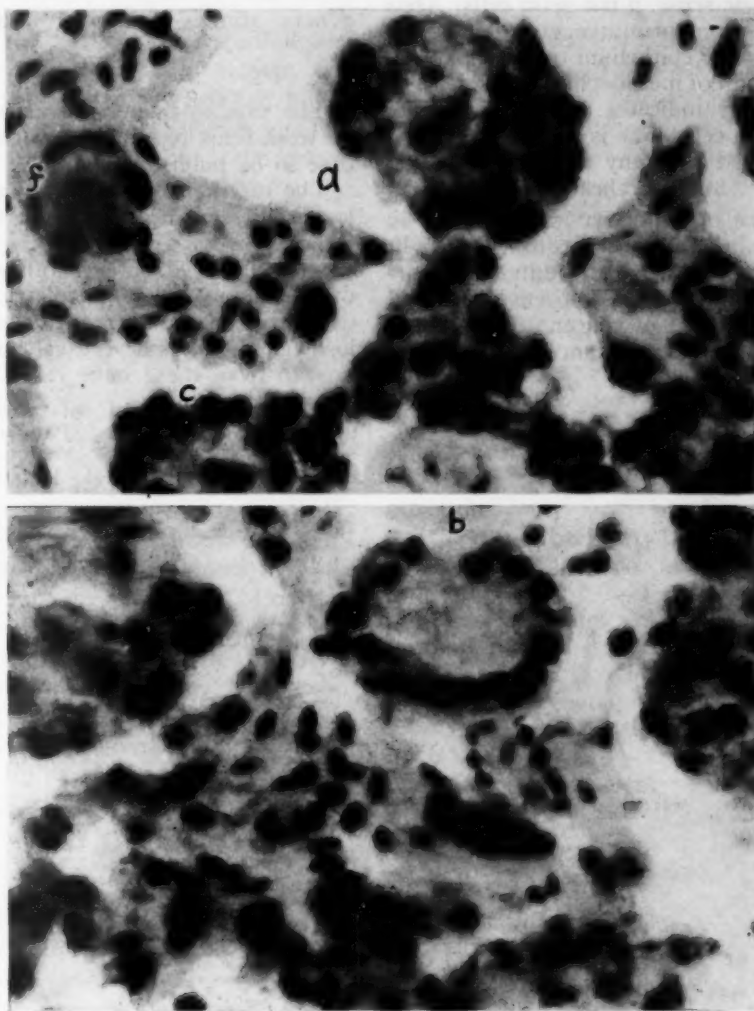

Fig. 2.—Details of the sections of granulosa cell tumor shown in figure 1; × 1,000; hematoxylin and eosin stain. See legend of figure 1 for an explanation of the letters *a, b, c* and *f*.

tissue slides I am prepared to side with the view that the tumor originated from granulosa cells. If this is so, one can offer the following hypothesis: Granulosa cells give rise to two tumors—(1) granulosa cell tumor, which is relatively differentiated, and (2) dysgerminoma, which is anaplastic and relatively undifferentiated. This theory, of course, can be confirmed only by further observations of such tumors. Careful postmortem examination of fetal bodies with complete histologic studies may

gest that these embryonal cell rests gave rise to granulosa cell tumors, and this idea was supported by R. Meyer, who held that adult membrana granulosa is incapable of producing tumors.

The present case would seem to indicate that mature, resting granulosa cells can give rise to tumors, contrary to the statement that no one has been able to trace the origin of any tumor in human subjects to the granulosa cells.[10]

On the other hand, Ewing[11] cited Furth and Butterworth, who irradiated the ovaries of mice,

thus inducing granulosa cell tumors, which were fifteen times as frequent as in the controls. Their histologic studies revealed that many of these tumors arose from adult follicles. Similar results were obtained by Geist [13] and his co-workers. This experimental work with animals is in agreement with the present observation of a tumor arising from granulosa cells.

It would serve no purpose here to delve into the controversial matter of the origin of granulosa cells. They are female sex cells in a histologic and a biologic sense, if not germ cells. They may be derived from primitive gonadal mesenchyme, from celomic epithelium or perhaps even from the epithelium of mesonephric tubules. The literature definitely indicates that the origin of ova and granulosa cells alike is not settled. This matter is discussed by many authors, including Ewing,[11] Novak [10] and Morehead and Bowman.[14]

The occurrence of neoplasia in childhood, in infancy and now in fetal life seems to lend weight to the opinion of those who believe in the importance of hereditary as opposed to environmental factors in the causation of cancer. It seems to indicate that at least some cancers may occur without the operation of extrinsic environmental factors.

On the other hand is the fact that the great bulk of cancers occur in the later decades of life.

The author believes in the theory that neoplasia is due to either germ cell or somatic cell mutations and that there are probably multiple and complex factors involved in mutation—some of which are now known—with others remaining to be discovered, as indicated by the significant genetic studies of Strong [15] and others.

SUMMARY

The case of bilateral ovarian carcinoma in a 30 week fetus reported is probably the first such case to be published.

The carcinoma appeared to arise from granulosa cells, but it had the morphologic characteristics of dysgerminoma.

It is suggested, subject to later correction, that dysgerminoma is an anaplastic undifferentiated derivative of granulosa cells and that the granulosa cell tumor is a relatively well differentiated growth from these same cells.

The foregoing clinical data have been given with the permission of Dr. F. J. Pearson.

13. Geist, S. H.: Am. J. Obst. & Gynec. **30**:650, 1935.

14. Morehead, R. P., and Bowman, M. C.: Am. J. Path. **21**:53, 1945.

15. Strong, L. C.: Arch. Path. **39**:232, 1945.

Obituaries

WILLIAM CRAMER
1878-1945

Dr. William Cramer was working with the great physiologist, A. E. Shafer, in Edinburgh, Scotland, when first I had the privilege of meeting him in 1913. Shafer then expressed himself as gratified to have in his department of physiology such an excellent chemist. In the nine years which Cramer spent with Shafer he evidently acquired skill in the organization and conduct of physiologic experimentation and judgment in making conclusions justified by the results. Thus were his chemical studies vitalized and brought to bear on function. It is the custom in British universities for microscopic anatomy, which we call histology, to be included in departments of physiology. In this line also Shafer was a master, and so Cramer entered the field of cancer strong in chemistry, in methods of physiologic experimentation and in knowledge of the minute structure of living tissues and organs —just the right mental equipment for his splendid work.

Even before Cramer went to Edinburgh he must have felt the challenge of the great riddle of cancer, because he had served for two years on the scientific staff of the Imperial Cancer Research Fund in London (1903-1905). While in Edinburgh he concentrated on the biochemistry of normal growth and metabolism, thus obtaining an excellent background for the investigation of cancerous growth. To the cancer problem he dedicated his life, as is evidenced by a series of publications extending over forty years. The very best place to work was in the laboratories of the Imperial Cancer Research Fund in London, to which he returned in 1915. Here the discovery by Yamagiva and Ichikawa, in 1916, that cancer can be produced in rabbits' ears by repeated applications of tar created a sensation. It marked the opening up of cancer as an experimental problem. During twenty-four years Cramer attained eminence in cancer research, he gave inspiration to many and he kept himself in good condition by repeated expeditions to the mountains, which he loved, in company with his devoted wife and later with his sons. Responsibilities increased, and Cramer was chosen time after time to serve as official British government delegate at various international congresses on cancer.

At the last congress in Atlantic City in 1939 he again met and immediately recognized me, this after an interval of twenty-six years. He had his wife and younger son with him, his elder son being in the British army. Owing to the war, the Imperial Cancer Research Fund advised him not to return to England, but to continue his researches on cancer in the United States, it being understood of course that the Fund would continue paying his pension as long as he lived. Fortunately, at this time an anonymous donor came to the support of the major cancer research project of the Barnard Free Skin and Cancer Hospital, St. Louis, and, realizing at once how greatly Cramer could help, he had made his contributions sufficient to provide for Cramer and an assistant. The purpose of the project was, and is, to discover exactly which properties of epidermis change and which remain constant as cancer develops in it in consequence of repeated applications of methylcholanthrene. Cramer brought all his wonderful training to bear on this problem and, with the help of his assistants, first R. E. Stowell and later W. L. Simpson, made important progress in standardizing the dosage, in tracing the carcinogen into the epidermis by its fluorescence, in observing the peculiar behavior of mast cells and in other particulars.

Mutually helpful cooperation has long existed between Barnard Free Skin and Cancer Hospital and Washington University, and so it was natural for Cramer also to be appointed research associate in cytology in the university. Although his work was centered in the hospital, his influence for good was felt by both faculty and students in the university, and the occasional lectures which he gave will long be remembered.

Space does not permit a review of the accomplishments of this leader in cancer research. Since the opening of the campaign, by the already mentioned introduction of experimental methods, he has been in the thick of the fight, advancing step by step, always constructively dealing with fundamentals—growth, normal and cancerous, the influence of vitamins and hormones, the effects of roentgen ray and radium, in which he made pioneering studies—his record stands as his monument. There was something solid about Cramer and his experiments which it is difficult to put into words. He did not pursue the policy of attracting attention by advancing revolutionary ideas or by creating needless controversy. He always seemed to have a reserve of facts to back up his statements, and he was meticulously accurate. Living most of his life in London, he occupied a strategic position in the medical world, receiving visitors from far and

near. There must have been many exciting moments when new vistas of knowledge of cancer opened up and also disappointments which he probably took with good humor in his stride. We cannot tell how closely he penetrated to the secret organization of the cancer cell because we remain ignorant of what that organization is.

sensitivity of animals to carcinogens which raised the whole question of the possibility of the existence of states of hypersensitivity to cancer in human beings. Plans for further experiments had been made. Moreover, he had almost completed the manuscript of a book on cancer detailing in a critical way his mature and far reaching
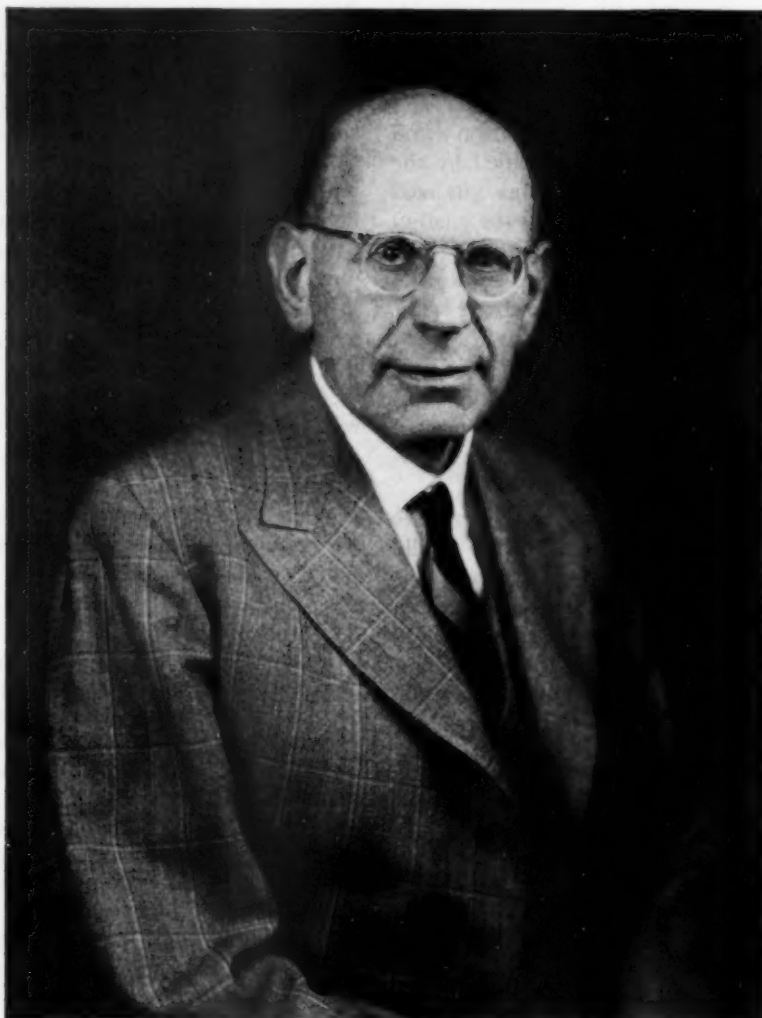
WILLIAM CRAMER, M.D.
1878-1945

But a lifetime of unremitting effort left him with unshakable confidence that the goal will be achieved, even though it now must be by others.

Until a few months before his death Cramer was thoroughly enjoying the life of his choice and working hard. Perhaps at no time was he more productive. He and Simpson had just discovered an experimental means of increasing the

knowledge of the problem. He spoke of it as his magnum opus giving everything he had so that others could have a good start.

Cramer died from cardiac complications, but autopsy showed an altogether unsuspected and inoperable cancer of the pancreas. Had the cardiovascular condition developed more slowly, he probably would have been claimed by the

mysterious disease which he knew so much better than most of us. His vision led him to realize that adequate treatment might be discovered if only we knew why some cancers grow rapidly, others slowly, and some change their rate of growth, enter into a phase of latency and in rare cases even disappear. For this reason he was in his last year deeply interested in the case of Sister Gertrude, who had an inoperable cancer of the pancreas (the diagnosis of carcinoma was established by laparotomy and biopsy). Sisters of the Order of St. Vincent de Paul interceded for her life with Mother Seton, the founder of the order; her symptoms disappeared and she lived for six years, dying of pulmonary embolus without any trace at autopsy of the original cancer. Cramer's associate,

Major G. Seelig, was expert at the Apostolic Court which was convened in New Orleans to determine the validity of these observations. Great achievement, much joy and happiness, and eventual tragedy mark the life of this man who was born in Brandenburg, Germany, June 2, 1878 and who died in Denver, Aug. 10, 1945.

The many friends of Cramer both in the United States and abroad will wish to know that Mrs. Cramer, who helped him so steadfastly in the path to greatness, as he did her in the field of art, expects to remain in St. Louis until her younger son, Michael, graduates from Washington University and her elder son, Captain Ian Cramer, joins her after his discharge from the British Army.

E. V. COWDRY.

Notes and News

Appointments, Etc. — At Marquette University School of Medicine, Milwaukee, W. A. D. Anderson, formerly associate professor of pathology in St. Louis University, has been appointed professor of pathology and bacteriology, and S. B. Pessin has been appointed associate professor of pathology and bacteriology.

I. N. Dubin, Duke University, has been appointed assistant professor of pathology and bacteriology in the University of Tennessee College of Medicine.

E. D. Warner has been appointed professor of pathology in the University of Iowa, succeeding H. P. Smith, now at the head of the department of pathology of Columbia University (College of Physicians and Surgeons), New York.

Hubert W. Smith, research associate in the medical and law schools of Harvard University, has been appointed professor of legal medicine in the graduate school of the University of Illinois.

E. C. Rosenow, professor emeritus of experimental bacteriology, Mayo Foundation, University of Minnesota, has accepted the post of bacteriologist at the Longview State Hospital, Cincinnati.

At the Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C., R. P. Morehead, associate professor of pathology, has been made director of the department of pathology and bacteriology in place of C. C. Carpenter, who will continue to be dean of the school and member of the pathologic staff as professor of legal medicine and director of clinical pathology.

Deaths. — William Cramer, pathologist and investigator of cancer, Barnard Free Skin and Cancer Hospital, St. Louis, died August 12, in his sixty-seventh year.

Pio del Río Hortega, Spanish histologist of the nervous system and disciple of Ramon y Cajal, died in Buenos Aires, Argentina, on June 1. He described microglia, oligodendroglia and neuroglia. This work and that on many other histologic aspects of the nervous system are well known.

Joseph McFarland, professor emeritus of pathology of the University of Pennsylvania, died September 22, at the age of 77 years.

Fellowships in the Medical Sciences. — Fellowships similar to those administered by the National Research Council since 1922 will again be available for the year beginning July 1, 1946. These fellowships, supported by the Rockefeller Foundation, are designed to provide for training and experience in research in all branches of medical science. They are open to citizens of the United States or Canada who possess the degree of M.D. or that of Ph.D. and are intended for recent graduates who are not yet professionally established.

The Council also administers two groups of research fellowships supported by the National Foundation for Infantile Paralysis. The first group, open to applicants who hold either of the degrees Ph.D. and M.D., is to provide for special training and experience in the study of filtrable viruses. The second group, open only to medical graduates who have completed one or more years of hospital experience in the practice of clinical surgery and are planning a career in orthopedic surgery, is designed to provide for training and research in those basic medical sciences that will be of particular value in furthering progress in orthopedic surgery.

A series of fellowships in anesthesiology has been established by the American Society of Anesthesiologists. These fellowships are offered to foster a closer union between the practice of anesthesiology and the fundamental disciplines on which anesthesia rests. Applicants must hold the M.D. degree and must have completed one or more years of hospital experience as intern or resident.

Fellows will be appointed late in February 1946. Applications to receive consideration at this time must be filed on or before January 1. Appointments may begin on any date determined by the board.

For further particulars address the Secretary of the Medical Fellowship Board, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

Books Received

TEXTBOOK OF OBSTETRICS: DESIGNED FOR THE USE OF STUDENTS AND PRACTITIONERS. By Henricus J. Stander, M.D., F.A.C.S., professor of obstetrics and gynecology, Cornell University Medical College, obstetrician and gynecologist in chief, New York Hospital, and director of the Lying-In Hospital, New York. Stander's third revision. Pp. 1277, with 740 illustrations. Price $10. New York and London: D. Appleton-Century Company, Inc., 1945.

This edition represents the latest of nine editions of Williams' Obstetrics, the first six of which were written by the late Dr. J. Whitridge Williams, professor of obstetrics, Johns Hopkins University School of Medicine, and obstetrician in chief to the Johns Hopkins Hospital, Baltimore. Attention is directed to this important textbook because its sections on the pathology of pregnancy, of labor, of the puerperium, of the fetus and of the newborn contain a great deal about matters of special interest to pathologists. The illustrations are good and there are extensive lists of references.

TEXTBOOK OF BACTERIOLOGY. By Edwin O. Jordan and William Burrows. Fourteenth edition. Price $7. Philadelphia: W. B. Saunders Company, 1945.

The edition has been thoroughly revised, and a good deal of new material has been added. The chapters on yeasts, molds and ray fungi have been replaced by a comprehensive new chapter on medical mycology. The chapter on parasitic protozoa (R. J. Porter) has been expanded to cover also flukes, tapeworms and roundworms. New maps and tables have been added. Most of the illustrations are new and original, serving their purpose well. The continued usefulness of this valued book is assured.

AN INTRODUCTION TO MEDICAL SCIENCE. By William Boyd, M.D., M.R.C.P. (Edin.), F.R.C.P. (Lond.), Dipl. Psych., F.R.S (Canada), professor of pathology and bacteriology, University of Toronto, and pathologist to the Winnipeg General Hospital, Winnipeg, Canada. Third edition, thoroughly revised. Pp. 366, with 126 illustrations. Price $3.50. Philadelphia 6: Lea & Febiger, 1945.

THE ROCKEFELLER FOUNDATION: ANNUAL REPORT, 1944. Pp. 344, illustrated. New York: Rockefeller Foundation, 1945.

CLINICAL BIOCHEMISTRY: By Abraham Cantarow, M.D., professor of physiological chemistry, Jefferson Medical College, formerly associate professor of medicine, Jefferson Medical College, and assistant physician, Jefferson Hospital; and Max Trumper, Ph.D., Lieutenant Commander, H(S), USNR, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md., and formerly in charge of the Laboratories of Biochemistry of the Jefferson Medical College and Hospital. Third edition, revised. Pp. 647, with 29 illustrations. Price $6.50. Philadelphia and London: W. B. Saunders Company, 1945.

HOSPITAL FOR JOINT DISEASES, NEW YORK. Thirty-Eighth Annual Report—For the Year 1944. Pp. 99, illustrated. New York: Hospital for Joint Diseases, 1944.

THE JOHN CRERAR LIBRARY, 1895-1944: AN HISTORICAL REPORT PREPARED UNDER THE AUTHORITY OF THE BOARD OF DIRECTORS BY THE LIBRARIAN. Pp. 206, illustrated. Chicago: The John Crerar Library, 1945.

THE BARNARD FREE SKIN AND CANCER HOSPITAL: SCIENTIFIC CONTRIBUTIONS, 1906-1944. Pp. 61. St. Louis: The Barnard Free Skin and Cancer Hospital, 1945.

ESSENTIALS OF HISTOLOGY. By Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., departments of anatomy of the Graduate School of Arts and Science and College of Dentistry, New York University. Price $3.50. Pp. 240, with 137 illustrations. St. Louis: The C. V. Mosby Company, 1945.

CANCER OF THE COLON AND RECTUM: ITS DIAGNOSIS AND TREATMENT. By Fred W. Rankin, B.A., M.A., M.D., LL.D., Sc.D., F.A.C.S., surgeon, St. Joseph's and Good Samaritan hospitals, Lexington, Ky., and A. Stephens Graham, M.D., M.S. (in Surgery), F.A.C.S., surgeon, Stuart Circle Hospital, Richmond, Va., and assistant professor of surgery, Medical College of Virginia. Second printing. Pp. 358, with 133 illustrations. Price $5.50. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

HAEMOGLOBIN LEVELS IN GREAT BRITAIN IN 1943 (WITH OBSERVATIONS UPON SERUM PROTEIN LEVELS). By the Committee on Haemoglobin Surveys. Medical Research Council, Special Report Series no. 252. Price 60 cents. London: His Majesty's Stationery Office (New York: British Information Service), 1945.

THE FIFTY-NINTH AND SIXTIETH ANNUAL MEDICAL REPORTS OF THE TRUDEAU SANATORIUM AND THE THIRTY-NINTH AND FORTIETH MEDICAL SUPPLEMENTS, 1943-1944, TOGETHER WITH THE ABSTRACTS OF THE STUDIES OF THE EDWARD L. TRUDEAU FOUNDATION FOR RESEARCH AND TEACHING IN TUBERCULOSIS. Pp. 40. Saranac Lake, N. Y.: Edward L. Trudeau Foundation, 1945.

PULMONARY EDEMA AND INFLAMMATION: AN ANALYSIS OF PROCESSES INVOLVED IN THE FORMATION AND REMOVAL OF PULMONARY TRANSUDATES AND EXUDATES. By Cecil Drinker, M.D., D.Sc., professor of physiology, School of Public Health, Harvard University, Boston. Pp. 106, illustrated. Price $3. Cambridge, Mass.: Harvard University Press, 1945.

DOCTORS AT WAR. Edited by Morris Fishbein, M.D., editor of *The Journal of the American Medical Association* and of *Hygeia, the Health Magazine,* chief editor of *War Medicine,* and chairman of the Committee on Information of the Division of Medical Sciences of the National Research Council. Pp. 418, with 82 illustrations. Price $5. New York: E. P. Dutton & Co., Inc., 1945.

THE FUNDAMENTALS OF ELECTROCARDIOGRAPHIC INTERPRETATION. By J. Bailey Carter, M.D., F.A.C.P., assistant (Rush) professor, Department of Medicine, University of Illinois College of Medicine; member of

the attending staff, Cook County Hospital and Augustana Hospital, Chicago. Pp. 406, with 307 illustrations. Price $6. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

THE ROCKEFELLER FOUNDATION: A REVIEW FOR 1944. By Raymond B. Fosdick, president of the Foundation. Pp. 63, illustrated. New York: The Rockefeller Foundation, 1945.

PULMONARY TUBERCULOSIS IN THE ADULT: ITS FUNDAMENTAL ASPECTS. By Max Pinner, M.D., chief of the division of pulmonary diseases, Montefiore Hospital for Chronic Diseases, New York; clinical professor of medicine, College of Physicians and Surgeons, Columbia University, New York. Price $7.50. Pp. 579, with 59 illustrations. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

ANNUAL REPORT FOR THE YEAR ENDED MAY 31, 1945. THE NATIONAL FOUNDATION FOR INFANTILE PARALYSIS, INC. Pp. 46. New York: National Foundation for Infantile Paralysis, Inc., 1945.

RECENT ADVANCES IN NEUROLOGY AND NEUROPSYCHIATRY. By W. Russell Brain, M.A., D.M. (Oxon.), F.R.C.P., physician to the London Hospital and the Maida Vale Hospital for Nervous Diseases, and E. B. Strauss, M.A., D.M. (Oxon.), F.R.C.P., physician for psychologic medicine, St. Bartholomew's Hospital, and honorable research psychiatrist, Maida Vale Hospital for Nervous Diseases. Fifth edition. Pp. 363, with 32 illustrations. Price $5. Philadelphia: The Blakiston Company, 1945.

TUBERCULOSIS IN THE UNITED STATES: GRAPHIC PRESENTATION. Prepared by the staffs of the Division of Public Health Methods and the Field Studies Section of the Tuberculosis Control Division, United States Public Health Service. Under the direction of Carroll E. Palmer, M.D. Volume 2: Proportionate Mortality Statistics for States and Geographic Divisions by Age, Sex and Race. Volume 3: Mortality Statistics for Cities of 100,000 or More Population by Age, Sex and Race, 1939-41. Price $1.50. New York: National Tuberculosis Association, 1944 and 1945.

CORRECTION

In the last sentence of paragraph 5 under "Notes and News" in the August issue "pathology" should read "anatomy"; in other words, Dr. Norris has been appointed visiting professor of anatomy in Washington University.